In Vitro Evidence That Urine Composition Affects the Fraction of Active Furosemide in the Nephrotic Syndrome

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ABSTRACT
Diuretic resistance to furosemide in the nephrotic syndrome (NS) may result from binding of drug to filtered albumin within the renal tubule. In buffer solutions intended to partially mimic the luminal environment of the distal nephron during the NS, we examined several chemical properties to determine their effect on furosemide-albumin binding equilibria. Dissociation constants were obtained by measuring furosemide's quenching of human serum albumin's intrinsic tryptophan fluorescence over ranges of pH, ionic strength (IS) and osmolality. Neither pH nor osmolality significantly affected binding; however, incremental increases in IS between 0.0 and 1.0 produced increases in Kd from 0.85 ± 0.05 to 34.38 ± 1.72 μM, resulting in a 5- and 28-fold increase in the unbound furosemide fraction when the furosemide-albumin concentrations were 3.0:5.0 and 10.0:45.0 μM, respectively. Our results indicate that human serum albumin contains one high affinity binding site for furosemide that is sensitive to IS. Because of changes in the concentrations of reactants as well as IS that can occur in nephron segments distal to furosemide's site of action, we conclude that the amount of unbound (i.e., pharmacologically active) drug in voided urine will not necessarily correspond to the amount at the active site. To clinically assess the pharmacodynamic consequence of protein binding in the NS, changes in the concentration of the reactants and IS in the distal nephron must be minimized so that the unbound furosemide measured in voided urine will accurately reflect the amount at the drug's active site.

The physiologic activity and the pharmacokinetic behavior of a number of ligands are modulated by the interaction of the ligand with proteins. The affinity between protein and ligand is typically examined under conditions representative of plasma; however, the activities of many drugs reside in extra-vascular compartments. Although binding isotherms in these nonvascular compartments are seldom measured, protein binding in the region containing the xenobiotic's active site would be an important determinant of drug response inasmuch as the pharmacologic active drug component is presumably limited to the unbound or free fraction. Diuretic resistance in the NS illustrates this general point. Furosemide, the most frequently administered loop diuretic, is 98% bound to albumin in serum (Andreasen and Jakobsen, 1974; Prandota and Pruitt, 1975; Cutler and Blair, 1979). However, this serum protein binding may be of secondary importance in the NS, because furosemide's diuretic activity is dependent upon delivery of free drug into the urine (Brater, 1978; Chennavasin et al., 1979) with subsequent inhibition of active NaCl transport in the TALH (Schlatter et al., 1983). Due to the low concentration of albumin normally present in urine, presumably all luminal furosemide exists in the free form. Patients with the NS, however, excrete large amounts of serum proteins, particularly albumin, into the urine and the binding of furosemide to albumin within the renal tubular lumen could reduce the free drug concentration at the active site and contribute to the subnormal diuretic response often observed in nephrotic patients.

Despite attempts by investigators to establish the mechanism of diuretic resistance in the NS (Rane et al., 1978; Green and Mirkin, 1981; Keller et al., 1982; Smith et al., 1985), the underlying cause, including the role of protein binding in the urine, remains poorly defined. Compared to normal subjects, nephrotic patients receiving loop diuretics often demonstrate a rightward shift in the "dose"-response relationship. That is, when the excretion rate of Na⁺ is plotted as a function of the excretion rate of furosemide, a supranormal amount of drug is needed to achieve a given level of response. If binding is clinically important, the abnormal dose-response curve should normalize when the dose is expressed as the excretion rate of free rather than total drug. Because in vivo studies both dose

ABBREVIATIONS: NS, nephrotic syndrome; TALH, thick ascending limb of Henle; IS, ionic strength; OSM, osmolality; HSA, human serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-performance liquid chromatography; CD, collecting duct.
ions in intensity upon serial additions of furosemide is directly bound to the acceptor pair (Stryer, 1978). Because the efficiency of Förster type energy transfer is dependent upon the distance between the donor and acceptor pair (Stryer, 1978), it appears that energy transfer only occurs to that fraction of furosemide bound to albumin. Therefore, the net decrease in the fluorescence intensity upon serial additions of furosemide is directly proportional to the fraction of albumin molecules that contain furosemide, thus forming the basis of a binding assay. The fortuitous high efficiency of energy transfer, leading to a near complete quenching of signal, facilitates the quantitation of the equilibrium binding constants.

The influence of the above mentioned variables on binding were determined in triplicate at 37°C by measuring the quenching after each serial 1.0 to 5.0 μl additions of the 3.0 mM furosemide solution to 2 ml volumes of the 5.0 μM HSA solutions. IS was tested by adjusting the

Methods

Fluorescence Experiments

Reagents. Furosemide powder (a gift from Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ), was used as received. A 3.0 mM stock solution in 20 mM HEPES buffer (Sigma Chemical Co., St. Louis, MO) was made by dissolving furosemide in buffer at pH 11.0, and once in solution lowering the pH to approximately 8.0 for the experiments. Fraction V fatty acid free HSA (Sigma Chemical Co.) was purified by sephadex chromatography and dialyzed overnight in 20 mM pH 7.0 HEPES buffer. Experiments involving fluorescence were conducted using 5.0 μM HSA (0.33 g/l, assuming MW 66,500). Before each experiment furosemide and albumin concentrations were confirmed by UV absorbance spectroscopy (model DU-65 Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA). The HSA concentration was measured using the 1 cm path length extinction coefficient ε280 of 5.3 at 280 nm (Sudlow et al., 1975) and furosemide by the molar extinction coefficient of 4830 M⁻¹ cm⁻¹ at 330 nm (determined in our laboratories). NaCl, KCl, CaCl₂, and urea (Fisher Scientific, Springfield, NJ) solutions were prepared in 20 mM HEPES buffer. All solutions were made at 37°C.

Binding titration curves. The effect of IS, pH and OSM on the binding affinity between furosemide and albumin was examined using fluorescence spectroscopy (MPF-66 Fluorescence Spectrophotometer, Perkin-Elmer, Norwalk, CT). This technique permits examination of the binding isotherm without perturbing the thermodynamics of the system under investigation and is applicable because the binding of furosemide to HSA quenches the fluorescence by the single tryptophan residue of HSA. A strong overlap exists between tryptophan’s emission and the absorbance spectrum of furosemide, suggesting that the quenching mechanism could be due to Förster type resonance-energy transfer, i.e., nonradiative energy transfer. The efficiency of this energy transfer appears extremely high as evidenced by virtually complete quenching of tryptophan’s emission by the addition of furosemide (fig. 1). Because the efficiency of Förster type energy transfer is dependent upon the distance between the donor and acceptor pair (Stryer, 1978), it appears that energy transfer only occurs to that fraction of furosemide bound to albumin. Therefore, the net decrease in the fluorescence intensity upon serial additions of furosemide is directly proportional to the fraction of albumin molecules that contain furosemide, thus forming the basis of a binding assay. The fortuitous high efficiency of energy transfer, leading to a near complete quenching of signal, facilitates the quantitation of the equilibrium binding constants.

Results

The influence of the above mentioned variables on binding were determined in triplicate at 37°C by measuring the quenching after each serial 1.0 to 5.0 μl additions of the 3.0 mM furosemide solution to 2 ml volumes of the 5.0 μM HSA solutions. IS was tested by adjusting the

Fig. 1. Quenching of the fluorescence of HSA by furosemide. Binding of furosemide to HSA quenches the protein’s single tryptophan emission. The binding isotherm of this reaction can be determined from the steady-state fluorescence from a solution containing HSA after serial additions of furosemide. The curve depicted exemplifies the hyperbolic relationship between the fluorescence intensity from 5.0 μM HSA and the furosemide concentration.

Fig. 2. Effects of IS on Kd. Fluorescence quenching of the intrinsic emission of HSA by serial additions of furosemide was measured in solutions of differing IS. Results are for experiments conducted at 37°C and pH 7.0, where IS was varied using NaCl, KCl or CaCl₂. For each ion pair, estimates of Kd ± S.E. for the furosemide-HSA complex represent a triplicate set of experiments. Also shown is each group’s mean ± S.D.
HSA solutions to either 0.0, 0.1, 0.5 or 1.0 with the appropriate molar amounts of NaCl, KCl or CaCl\(_2\). Osmotic effects were tested on the HSA solutions containing either 0, 10, 100 or 250 mM urea; pH of the final solutions was adjusted to 7.0 or 8.5 with HCl and NaOH.

An excitation wavelength of 300 nm was chosen to avoid excitation of the tyrosine residues; the 300 nm excitation and 370 nm emission wavelengths minimized the inner filter effects by furosemide. Excitation and emission slit widths of 1.5 and 10 nm, respectively, were used. In the absence of furosemide, the emission intensity was determined to be linearly dependent upon concentration of albumin. The small dilution effect on the observed intensities resulting from furosemide addition was taken into account. The pH of solutions was not affected by the addition of furosemide, and the solutions were exposed to exciting light only for the few seconds necessary for each measurement to minimize any possible photochemical events. The absorption of exciting or emitted light by furosemide and albumin was negligible at the concentrations and wavelengths utilized so that no inner filter effects were observed. The absence of these inner filter effects was also verified directly by performing some titrations using front-face observations with a SPEX photon counting fluorometer.

**Determination of \( K_d \)**. The curve describing the hyperbolic relationship between the steady-state fluorescence intensity of HSA and total furosemide concentration (fig. 1) was fitted via PC NONLIN to the equation

\[
I = I_0 \left( 1 - \frac{Q \cdot C_{u}}{K_d + C_{u}} \right)
\]

where \( I \) = fluorescent intensity in the presence of bound furosemide; \( I_0 \) = initial fluorescent intensity; \( Q \) = proportionality constant that equates the change in fluorescence intensity to the amount of bound furosemide; \( C_u \) = unbound furosemide concentration; and \( K_d \) = dissociation constant.

To obtain the best estimate of the true \( K_d \) for each experimental condition, all steady-state fluorescence intensity measurements from the triplicate set of experiments were entered together as a function of total furosemide concentration. A \( K_d \) value for furosemide-albumin reactions corresponding to the individual ion pairs at each IS tested was determined by a simultaneous least-squares iterative fit of equation 1 after substituting the equivalent of \( C_u \) in equation 2 for \( C_u \) in equation 1.

\[
C_u = \frac{1}{2} \cdot \frac{(C - K_d - P) + \sqrt{(P + K_d - C)^2 + 4 \cdot K_d \cdot C}}{2}
\]

\( C_u \) and \( K_d \) have the same meaning as in equation 1. \( P \) and \( C \) are the total protein concentration and total furosemide concentration, respectively. Both equations are modifications of the equation describing the law of mass action, assuming a single furosemide binding site on HSA.

**Estimates of unbound furosemide concentration**. Based on the \( K_d \) values obtained from the experiments with NaCl, estimates of unbound furosemide concentration were obtained from equation 2 for two sets of furosemide and albumin concentrations. Concentrations of 3.050.0 \( \mu \)M and 10.0-45.0 \( \mu \)M furosemide-HSA, respectively, were chosen because they are expected to encompass concentrations present in the distal nephron of nephrotic patients.
Ultrafiltration Experiments

To confirm a single binding site on HSA for furosemide and the accuracy of the unbound drug estimates for the various IS tested, ultrafiltration experiments with HPLC measurement of unbound drug concentration were performed using the two sets of furosemide and albumin concentrations. Preliminary experiments excluded significant binding of drug to membrane. Preparation of drug and protein with spectroscopic confirmation of concentration was performed in a manner similar to the fluorescence experiments. Experiments were performed in triplicate by adding HSA to solutions of NaCl for a final IS of 0.0, 0.1, 0.5 or 1.0. After adjusting these solutions to pH 7.0, furosemide was added (pH not affected by its addition), the mixtures were gently swirled and incubated at 37°C for 10 min. Although binding equilibrium is achieved on a much briefer time scale, the concentration of reactants was stable over this interval as verified by absorbance spectroscopy. Thus, values at 10 min are accurate and this time of incubation was operationally feasible. One milliliter of a solution containing HSA, furosemide and NaCl was added to a MFS-1 micropartition system containing a YMT membrane (Amicon Corp., Denvers, MA) and centrifuged at 37°C and 200 x g for 2 min. Furosemide concentration in the ultrafiltrate (unbound drug) was measured by HPLC using methods described previously (Chennavasin et al., 1979; Breter et al., 1984). Briefly, 30 μl of metolazone internal standard was added to 200 μl of ultrafiltrate. One hundred microliters of glacial acetic acid was next added to each sample followed by 5 ml of methylene chloride and reciprocal shaking for 30 min. The samples were centrifuged at 800 x g for 10 min. and the organic phase collected and evaporated to dryness at 37°C with N2 gas. Samples were reconstituted in 70% methanol and injected onto a Beckman CI8 reverse phase column (Beckman, San Ramon, CA). The column was eluted with 38% acetonitrile, in 0.5 M Na+ acetate buffer (pH 3.6) at a flow rate of 1.0 ml/min. The diuretics were detected fluorometrically with a 650-15 Fluorescence Spectrometer (Hitachi, Ltd., Tokyo, Japan) using excitation and emission wavelengths of 344 and 410 nm, respectively.

Results

Figure 2 demonstrates the curvilinear relationship between IS and the $K_d$ for each electrolyte pair tested. Increasing IS from 0.0 to 1.0 resulted in a rise in $K_d$ from 0.65 ± 0.5 to 34.48 ± 1.72 μM, a 53-fold change, and the relative effect was greatest at the lower values of IS. This relationship between IS and $K_d$ held at both pH 7.0 and pH 8.5 (and pH 5.5, data not shown). Except at 0.0 IS, pH did not affect binding affinity$^4$ (fig. 3). Similarly, OSM did not alter $K_d$ (fig. 4) and the values determined at each pH were numerically similar to the measurements shown in figure 3 at 0.0 IS.

From equation 2, values of $K_d$ yield unbound furosemide concentrations for any total concentration of furosemide and albumin. Figure 5 shows the expected free fraction for two albumin-furosemide solutions as a function of the $K_d$ values obtained when IS was varied with NaCl. The concentrations of furosemide and albumin are estimates of the total amounts that could occur within the distal nephron. Curve A in figure 5 depicts an approximate 5-fold change in free drug concentration along the range of IS tested when the albumin concentration was the same as in the fluorescence experiments. In comparison, curve B demonstrates a smaller absolute change in the unbound furosemide fraction over the same range of IS. The albumin:furosemide ratio in curve B, however, is larger than in A, resulting in greater binding and less free drug at zero IS. Hence, even though the absolute change is less than in curve A, a 28-fold difference in unbound fraction occurs between the two extremes of IS.

Figure 6 compares the unbound furosemide concentrations that correspond to the unbound fraction in figure 5 with the amounts measured by ultrafiltration, a semidirect method of measuring protein-ligand interactions. As can be seen, ultrafiltration could account for approximately 85% of the calculated binding from the fluorescence experiments. Furthermore, this relationship held for each set of furosemide-albumin solutions over the entire range of IS tested.

Discussion

By using fluorescence spectroscopy, we found an inverse relationship between IS and the binding of furosemide to HSA (fig. 2). This influence of IS on $K_d$ illustrates the dependence of the binding isotherm on the immediate environment and demonstrates that the binding affinity between the protein and ligand depends upon the conditions under study. In experiments concerned with drug pharmacology, binding is usually measured in an environment representative of plasma. Because of the relatively invariant plasma milieu, binding affinities in this compartment are essentially constant and environmental influences other than the concentrations of protein and ligand themselves are negligible. In contrast to plasma, changes in the composition and concentration of the various constituents of urine are extensive. Although any variability in the equilibria of a reaction due to normal alterations in the plasma environment are not likely to be clinically important, the amount of bound ligand in urine could be significantly affected by changes that normally take place within the renal tubule. Thus, because furosemide binds to albumin in the nephron of patients with the NS (Smith et al., 1985), our observation has implications that are important when examining the role of protein binding as a mechanism of diuretic resistance.

The resistance to diuretic drugs exhibited by nephrotic patients often results in difficulties in maintaining proper extracellular fluid volume. To design treatment strategies aimed at circumventing this problem, the importance of identifying the pathophysiology responsible for the drug resistance is obvious. Several investigators have examined the possible mechanisms of diuretic resistance in the NS (Rane et al., 1978; Green and Mirkin, 1981; Keller et al., 1982; Smith et al., 1985), but as yet the exact cause(s) remains unknown. Of the postulated mechanisms, the most intriguing emanates from a study in a rat model of the NS in which the authors found 60 to 95% of the excrated furosemide was bound to the protein contained in urine, and the chloriuretic response to the drug was inversely correlated to the urinary albumin excretion rate (Green and Mirkin, 1980). These results imply, but do not prove, that protein binding within the renal tubule reduces the active drug concentration and is a cause of resistance. The effects of protein binding on furosemide’s diuretic activity has also been examined in one study involving patients with NS (Smith et al., 1985). Although a high degree of furosemide binding to urine proteins was again found, this mechanism could not entirely account for the diuretic resistance inasmuch as a normalization of the dose-response relationship did not occur when the un-

$^4$ The true IS of the solution is not zero since proteins are polyelectrolytes. In addition, the initial pH of the solutions was acidic and 6 N NaOH was added to obtain the desired pH. Compared to the pH 7.0 solutions, the pH 8.5 solutions required approximately 10 to 15 mEq/l more NaOH to obtain the desired pH. We suspect the slightly greater IS of the pH 8.5 solutions to be the cause for the higher $K_d$ observed. The small differences in IS between the two solutions were insufficient to cause observable differences in $K_d$ when Cl− salts were added to raise the IS to 0.1 or greater.
bound component of urine furosemide was considered. The inability of this latter investigation to ascertain the importance of protein binding may be the result of an implicit assumption contained in the study; namely, that the measured amount of unbound furosemide in samples of voided urine represented the quantity present at the drug's TALH site of action. Based on our results, the assumption that furosemide's protein binding is constant along the nephron appears invalid and this error may have prevented an accurate assessment of the role played by protein binding in the diuretic resistance during the NS.

The objective of this study was not to evaluate the clinical importance of protein binding in the NS, but rather to determine what urinary factors must be considered when designing a clinical study that will properly evaluate this potential mechanism of diuretic resistance. We tested the effects of IS, pH and OSM on the binding of furosemide to HSA in amounts that would encompass at least portions of the ranges expected within the distal nephron. The effects on protein binding by IS between 0.0 and 1.0 are shown in figure 2. This range was chosen based on studies examining Na⁺ concentrations along the nephron because, in general, this ion is the most abundant electrolyte in urine. The greatest fluctuations in Na⁺ concentration occur in the distal nephron, although technical barriers have made it difficult to ascertain the precise concentrations in the deeper segments. Furthermore, the concentration will likely vary depending upon the species of animal and the conditions chosen for study. For example, free flow micropuncture studies in rat (Jamison et al., 1967) and hamster (Marsh, 1970) have found mean thin ascending limb Na⁺ concentrations of 257 and 800 mM, respectively. NaCl constitutes approximately 50% of the papillary solute and in humans, maximum urinary concentrating ability is 1200 to 1400 mOsmol/kg. Thus, when NaCl within the thin ascending limb reaches equilibrium with the adjacent papillary interstitium, during conditions producing a maximally concentrated urine, the intraluminal NaCl concentration would be expected to be roughly 300 to 350 mEq/l. Na⁺ is reabsorbed in the TALH and reports in the rat have shown early distal tubule concentration less than 50 mM (Good et al., 1984). Further reabsorption can take place in the distal tubule (Ellison et al., 1987) and CD (Hierholzer, 1985). The concentration can also be increased along the CD due to ADH-induced water reabsorption. Hence, depending on the condition of the subject, urine exiting the kidney can vary in Na⁺ concentration from a few millimolar to several hundred millimolar. In order to account for the contribution of other electrolytes and encompass the entire range of urinary IS, we conducted our IS experiments between 0.0 and 1.0. Although the normal IS of urine may not reach either 0.0 or 1.0, figure 2 shows that the relative effects of electrolytes on Kᵣ are greatest at the lower range of IS, values that clearly occur within the distal nephron.

The clinical significance of the effect of IS on furosemide's protein binding was estimated using equation 2 to calculate the free drug fraction (fig. 5). As can be seen in figure 5 and in accordance with the law of mass action, the effect of IS on the unbound fraction depends upon the total concentration of both reactants. Over the range of IS tested, a several-fold rise in the unbound furosemide concentration was demonstrated for the two sets of reactants. The relative magnitude of change is greatest at the lower values of Kᵣ and hence, IS. The concentrations of furosemide and HSA used in the calculations were intended to reflect amounts expected to be present in the distal nephron during the NS. Microperfusion studies on isolated TALH segments from rats have shown furosemide at 3.0 μM concentrations produces 50% inhibition of Na⁺ transport by this segment (Schlatter et al., 1983). Thus, the 3.0 and 10.0 μM furosemide concentrations used in this study would be obtainable in nephron segments inclusive of the TALH and beyond. Little is known about the concentration of albumin in the various nephron segments under nephrotic conditions. A micropuncture study in nephrotic rats has measured proximal tubular albumin concentrations between 2 and 14 mg/100 ml (0.3 and 2.1 μM) (Oken et al., 1972). The concentration in the distal nephron would expectably be higher due to water reabsorption in the remaining descending limb. Although protein concentrations used in this study may exceed the actual levels...
found in TALH, our focus was on events occurring beyond this segment as changes in binding in post-TALH segments may cause the unbound drug concentration in voided urine and the TALH to differ. The concentrations of 5.0 and 45.0 μM are obtainable in the CD and final urine. It should be noted that any water reabsorption in the CD will concentrate both reactants, with concomitant influences on binding. Because both IS and the concentrations of reactants contained in this study mimic in part the distal nephron during the NS, it appears that the magnitude of IS changes in relevant portions of the nephron are sufficient to produce significant changes in the unbound furosemide concentration. From a clinical standpoint, these results indicate that the changes that occur as urine traverses the nephron will cause the unbound drug concentration in the TALH and in voided urine to differ. Because solute reabsorption by nephron segments distal to the TALH is ongoing during a furosemide induced diuresis, assessment of binding in voided urine is unlikely to represent conditions at the drug’s site of action.

In addition to IS, we also tested the effects of pH and OSM on binding. HSA is known to contain a binding drug site that undergoes alterations in binding affinity in the range of pH 6.0 to 9.0 (Witting et al., 1980; Wanwimolruk and Birkett, 1982). The pH of final urine can vary between 4.5 and 8.0 (Cogan and Rector, 1986). Therefore, if furosemide were to bind to this site, pH changes in the final urine could affect unbound drug concentrations and accentuate differences in binding between the TALH and voided urine. Except for the solutions where Cl− salts were not added (0.0 IS) (fig. 3), we did not observe a pH effect on binding. These results, in conjunction with a lack of effect at pH 5.5, indicate that furosemide’s binding to HSA is not influenced by pH changes that encompass most of the range found in the distal nephron. Similarly, urine OSM does not seem to influence the binding of furosemide to albumin (fig. 4). Although no effects on binding were observed when osmotic testing with urea was conducted at concentrations between 0.0 and 250 mM, as mentioned, the osmolality of urine can exceed 1000 mOsmol/kg. It should be noted that there is osmotic activity from the various salts used in testing the effects of IS. The OSM of a solution containing the univalent ion pair NaCl or KCl is approximately twice the molarity of the solution. Inasmuch as the effect of NaCl and KCl on the unbound drug fraction (fig. 5 in conjunction with fig. 2) occurs primarily at concentrations less than or equal to 100 mM, then even if the influence of these electrolytes on drug binding were solely due to osmotic effects rather than IS, the actual impact of OSM on the unbound concentration would be minimal at the large K∞ values that correspond to the greater values of osmolality (i.e., high electrolyte concentrations). Overall, then, our data appear to exclude significant osmotic or pH effects on binding and indicate that IS is the major determinant of binding between albumin and furosemide.

Based on the hyperbolic relationship between furosemide concentration and fluorescence intensity (fig. 1), we assumed in our model a single furosemide binding site on HSA. Other studies under conditions unlike ours have also determined a second, lower affinity binding site (Andreasen and Jakobsen, 1974; Prandota and Pruitt, 1975; Parsons, 1983). For this reason, we performed comparative ultrafiltration experiments. As can be seen in figure 6, the unbound furosemide concentrations estimated from the fluorescence experiments are quite similar to the amounts measured by ultrafiltration for both sets of reactants at the tested range of IS. These results confirm both the accuracy of our model and the validity of our findings concerning the significance of IS on furosemide’s protein binding.

Because the various conditions tested in this study are expected to reflect the environment of distal nephron segments, we conclude that the unbound furosemide fraction in the voided urine of patients with the NS will not usually reflect the amount of free drug at the TALH active site. Although our results do not delineate the clinical importance of protein binding as a mechanism of diuretic resistance in the NS, they provide the initial step for the development of a definitive clinical study. Our data indicate that electrolyte and water transport in the nephron distal to the TALH must be minimized if one hopes to obtain clinically meaningful data that will allow a proper assessment of this potential mechanism of diuretic resistance.

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References


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