Kidney

DIURETICS: UPDATE ON MECHANISMS OF ACTION AND USES

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I. Introduction

The discovery and clinical application of diuretics is one of the most significant advances in twentieth century medicine. Diuretics are not only among the first synthetic drugs to be used clinically, but they are among the safest and most sophisticated therapeutic agents. Classically we think about the application of diuretics in the setting of edema or hypertension. However, it should be realized that diuretics are ion transport inhibitors and thus, either intentionally or unintentionally, have effects on nonrenal tissue. Some of the actions of diuretics on nonrenal tissue contribute to unwanted side effects but some extrarenal actions may be clinically useful. Advances in renal and ion transport research has led to the design and synthesis of a number of new diuretics, some of which are related to previous well-known diuretics and others which have unique structures and actions. This presentation will review certain basic cellular transport systems that are relevant to understanding the mechanism and site of action of diuretic drugs. The similarity between these renal transport systems and those found in other cells (i.e., red cells and vascular smooth muscle cells) will be presesented with speculation on how these similarities may relate to the extrarenal effects of diuretics--especially in the treatment of hypertension. Finally, certain new diuretic agents will be discussed including aquaretics--agents which primarily inhibit renal water absorption.

II. Basic Ion Transport Systems

As will become evident in the following discussion, the kidney serves as a useful model for virtually all the ionic cellular transport

systems that are found in cell biology. In the kidney, these transport systems are responsible for maintaining salt and water and acid-base homeostasis, while in other tissues they are critically responsible for normal processes such as cell volume regulation, cell proliferation, fertilization, motility, cell growth, and metabolic substrate uptake.

With respect to their action in the kidney, we are most concerned about the effects of diuretics on sodium and water absorption. It should be realized that, with rare exception, the effects of diuretics on sodium absorption in renal epithelia are localized to the luminal or apical cell membrane. Thus, to understand the cellular action of a diuretic, we should focus our attention on those mechanisms which are responsible for the movement of sodium from luminal fluid into renal epithelial cells.

The first major mechanism responsible for sodium entry into cells is depicted in Figure 1 and is the mechanism used predominantly by proximal tubule cells. Sodium entry in proximal cells is accomplished by a specific apical cell membrane proton exchanger or antiporter. Normal operation of this exchanger is dependent upon the maintenance of a lumen to cell [Na] gradient and maintenance of a lower urine to cell proton gradient. The former is maintained by the basolateral cell membrane sodium pump, Na ATPase which maintains intracellular sodium activity approximately 1μ10 that of luminal fluid. The latter is accomplished by rapid buffering of secreted protons by luminal bicarbonate and the catalyzed dehydration of the subsequently formed carbonic acid by the enzyme carbonic anhydrase. The enzyme carbonic anhydrase is also critically involved intracellularly in the generation

of the proton for which sodium is exchanged. In such a cell, diuretics that would inihibit sodium absorption must work either directly on the sodium proton exchanger, the enzyme carbonic anhydrase, or the enzyme sodium potassium ATPase. Since, at concentrations normally achievable in vivo, none of the diuretics directly block the sodium pump at the basolateral cell membrane, it is accurate to summarize that in this and all renal epithelial cells diuretics predominantly inhibit the access of luminal sodium to the basolateral sodium pump.

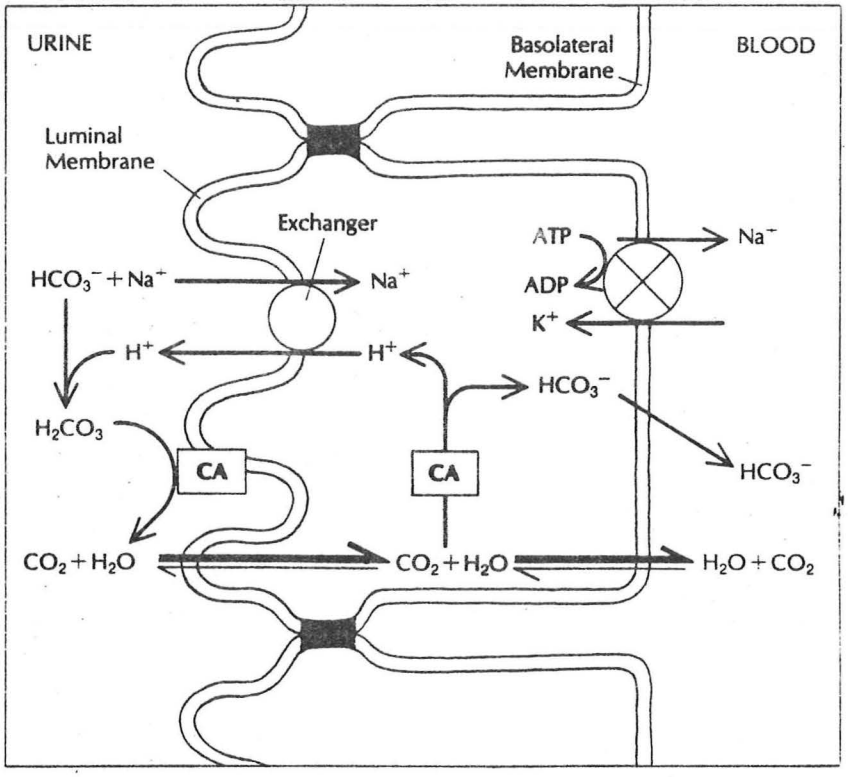

Figure 1. Schematic illustration of major transport events at apical and basolateral membranes of proximal tubule cells. See text for details.

The sodium proton antiporter in renal epithelial cells is responsible for net sodium and bicarbonate absorption while in most nonepithelial eukaryotic cells, it is a major mechanism for regulation of internal pH (3). The diuretic, amiloride, inhibits this sodium

proton exchanger but as we shall see later does not owe its diuretic activity to this mechanism. Very high concentrations of amiloride (1 ml) which are not achievable clinically inihibit proton exchange via two mechanisms. These mechanisms include amiloride competing for sodium at the sodium transport site of the exchanger and amiloride binding to a modifier site (4). It should be noted that recently several derivatives of amiloride have been synthesized and are significantly more potent than the amiloride in blocking the sodium proton antiporter (5). Because of the general distribution of sodium proton antiporters in most cells and their importance in regulation of cell pH, it is unlikely that potent inhibitors will find clinical usefulness as diuretic agents.

The second diuretic group which effects this apical cell membrane transport process is the carbonic anhydrase inhibitors. There is no evidence that these agents have a direct effect on the sodium proton antiporter. They owe their inhibition of sodium proton exchange to two mechanisms: 1) reduction in the availability of protons via intracellular action, and 2) accumulation of an acid disequilibrium pH in luminal fluid secondary to delayed dehydration of luminal carbonic acid. This latter process allows luminal pH to fall and thus provides a less favorable gradient for sodium driven proton excretion from proximal tubule cells (6).

While the sodium proton antiporter is a major mechanism for sodium entry into proximal tubule cells, there are other mechanisms for sodium entry which include sodium coupled glucose, amino acid, and organic anion transport, as well as an electroneutral sodium chloride absorptive process that may involve parallel sodium-proton exchange and chloride-

bicarbonate exchange (7). With the possible exception of parallel sodium-proton and chloride-bicarbonate exchangers, no clinically useful diuretic agents inhibit these additional apical cell membrane sodium entry steps. Also, since it has yet to be resolved how important the presence of apical chloride-bicarbonate exchange in parallel with sodium-proton exchange is in proximal tubule sodium chloride absorption, discussion of diuretic effects on this transport process in the proximal tubule is premature.

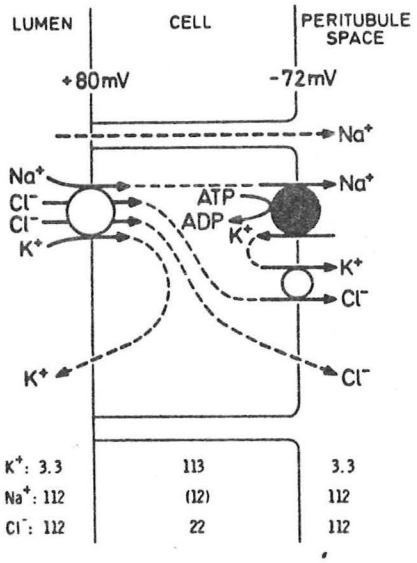

Figure 2. Schematic illustration of transport events at apical and basolateral membrane of thick ascending limb cells. See text for details.

The second major apical transport system of interest exists at the luminal surface of cells which line the thick ascending limb of Henle. Figure 2 illustrates such a cell. At the apical cell membrane is a complex electroneutral carrier that brings sodium, potassium, and two chlorides into the cell. The energy for this carrier mediated uptake is

provided by the basolateral cell membrane NaK ATPase pump which maintains intracellular sodium activity low (approximately 10 mM). The sodium which enters the cell via this carrier is transported to the blood via the sodium pump. The two chloride anions that are transported exit the cell into the blood via two different mechanisms. The first mechanism is an electroneutral KCl transporter. The second mechanism is conductive exit of chloride through a chloride channel. Due to the energy derived from the sodium gradient, the apical cell membrane carrier places intracellular chloride and potassium activity above equilibrium. Thus, there is an electrochemical gradient for chloride to enter the blood via a conductive pathway. There appears to be no major potassium conductance across the basolateral cell membrane, and therefore, potassium which enters the cell via the electroneutral carrier recycles into the urine across the apical cell membrane which exhibits very high potassium conductance. Movement of chloride from cell to blood contributes to the transepithelial positive potential observed in thick ascending limbs. An additional factor in the generation of a lumen positive potential is the recycling of potassium from the cell into the lumen. This transport system is very efficient because the electroneutral transport of sodium chloride generates a lumen positive potential via chloride and potassium diffusion out of the cell. The lumen positive potential is harnessed as a driving force for additional sodium movement from urine to blood through the paracellular space. For every sodium transported via the carrier transcellularly, an additional sodium is transported passively through the paracellular space (8-13).

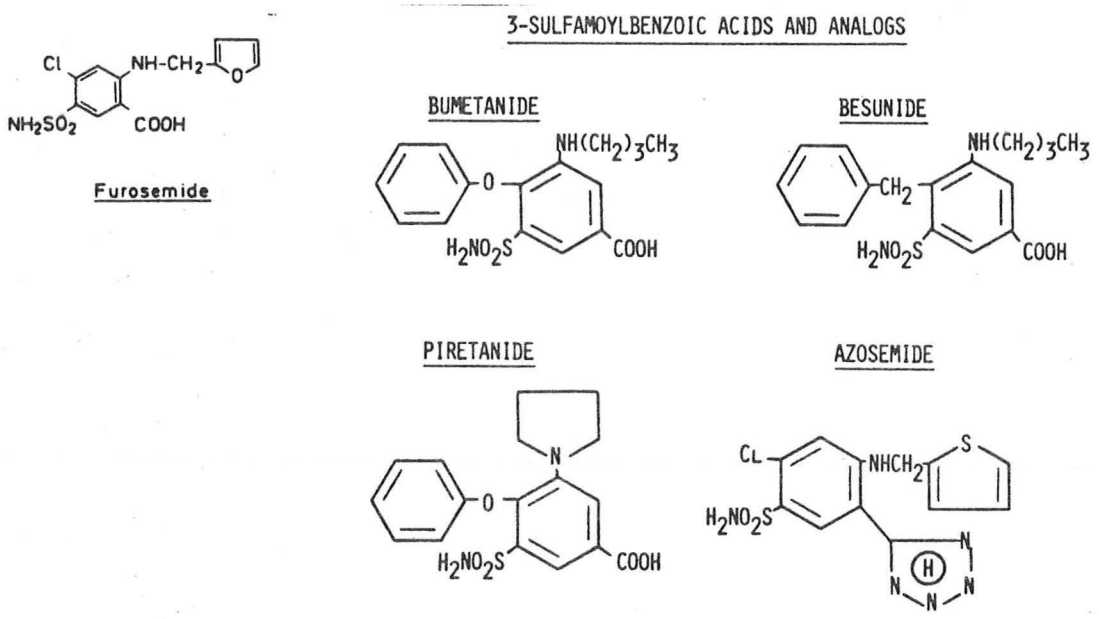

Figure 3. Structures of furosemide and 3-sulfamoylbenzoic acids.

Diuretics which act in the loop of Henle have several possible cellular mechanisms of action. Remembering that NaK ATPase inhibition is not a mechanism used by diuretics, candidates include the apical cell membrane carrier, the apical cell membrane potassium conductance, basolateral cell membrane potassium chloride cotransport, and the basolateral cell membrane chloride conductance. Drugs which are classified in the group called "loop diuretics" comprise a heterogeneous group including mercurial diuretics, phenoxyacetic acids, furosemide and related compounds, and newer agents such as muzolimine, L-ozolinone, tizolemide, and others. The structural differences among these compounds make it very likely that their cellular mechanisms of action differ.

Furosemide and other 3-sulfamoylbenzoic acids are illustrated in Figure 3. These compounds all appear to have in common binding to the apical cell membrane carrier, most likely at the chloride binding site

(14). These sulfamoylbenzoic acid analogues generally inhibit the carrier at concentrations from .1 to 10 μM. It should be noted that the sodium-potassium-2 chloride cotransporter is present in many tissues including intestine, gall bladder, cornea, certain cultured cell lines, shark rectal gland, Ehrlich ascites tumor cells, and red blood cells from various species (15-27). In most cells containing this transport system, the relative potency of the sulfamoylbenzoic acids are bumetanide > piretanide > furosemide.

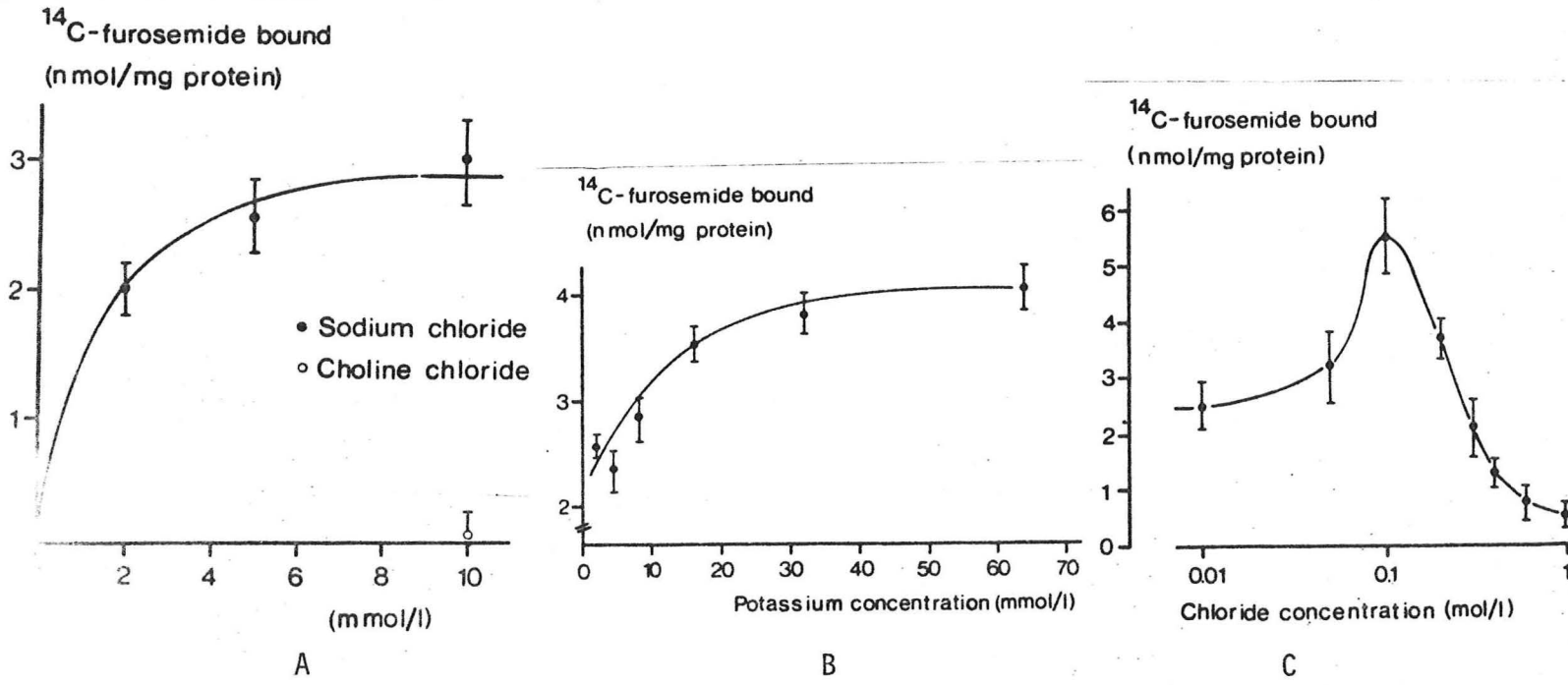

Figure 4. Effects of various Na, K, and Cl concentrations on furosemide binding to Tamm-Horsfall protein. See text for details.

There is some interesting evidence that the Tamm-Horsfall protein is related to the sodium-potassium-2 chloride transport system. Tamm-Horsfall protein is a renal epithelial glycoprotein which has been localized mostly to the apical cell membrane of the thick ascending limb of Henle. This protein has been isolated and purified from urine and

has a molecular weight of approximately 100,000 daltons (28-34). Figure 4A, B, and C illustrate certain characteristics of ^{14}C furosemide binding to Tamm-Horsfall protein. Figure 4A shows the furosemide binding/mg Tamm-Horsfall protein as a function of sodium chloride concentration. Binding was determined at 0°C after 30 minutes incubation of 80 μm furosemide in a medium containing 2.5 mg/ml of Tamm-Horsfall protein, 4 mM tris buffer at pH 8.0, and increasing concentrations of NaCl. Saturation of furosemide binding occurred at approximately 5 mM NaCl concentration. Proof that Na was responsible for this enhanced binding are the observations with 10 mM choline chloride where no significant binding was observed. Linear transformation of this binding curve shows that half-maximal furosemide binding occurs at a [Na] of 1.5 mM. Figure 4B illustrates the effects of [K] on furosemide binding to Tamm-Horsfall protein. These experiments were performed at 0°C after 30 minutes of incubation of 80 μM furosemide in a medium containing 2.5 mg/ml Tamm-Horsfall protein, 4 mM tris buffer pH 8.0, and 10 mM of sodium nitrate with increasing concentrations of potassium nitrate. Saturation of furosemide binding occurred at potassium concentrations above 40 mM. Linear transformation of this binding curve shows that half maximal saturation of furosemide binding occurs at 14 mM [K]. Figure 4C illustrates the effect of [Cl] on furosemide binding. These studies were done under similar conditions except the [Na] was maintained at 10 mM and the [Cl] increased by utilizing choline as the cation. Furosemide binding increased up to a [Cl] of 100 mM and subsequently decreased. The shape of the furosemide binding [Cl] relationship suggests the presence of positive coopertivity. It is

important to note that studies not graphically depicted demonstrated that loop diuretics with structure similar to furosemide, i.e. sulfamoylbenzoic acids were able to displace furosemide from its binding sites on Tamm-oHrsfall protein while loop diuretics with dissimilar structure could not (34).

Since the Na-K-2Cl cotransport system is present in so many cell types, identification of the Tamm-Horsfall protein with this transport system should be strengthened by finding Tamm-Horsfall protein in these other cell types. It is thus interesting to note that immunologic cross-reactivity has been demonstrated between Tamm-horsfall protein and erythrocyte ghosts (35). It should be pointed out however that a great deal of additional work is required before one can conclud that Tamm-Horsfall protein is directly related to the Na-K-2Cl cotransporter.

Furosemide and structurally similar compounds at higher concentrations, i.e. >100 μM can inhibit chloride hydroxyl exchange and KCl cotransport. It is likely that the other loop diuretics such as ethacrynic acid, indacrinone, L-ozolinone, and muzolimine which have been shown to effect transport from the blood side owe their diuretic effect, at least in part, to inhibition of KCl cotransport. This, of course, remains to be proven.

The next major mechanism for Na movement across the apical membrane is present in the true distal convoluted tubule. This mechanism involves the electroneutral coupling of Na and Cl entry as depicted in Figure 5. Shown in this figure is a coupled NaCl apical cell membrane entry step which is inhibited by furosemide. While very high concentrations of furosemide may inhibit this transport process, there is

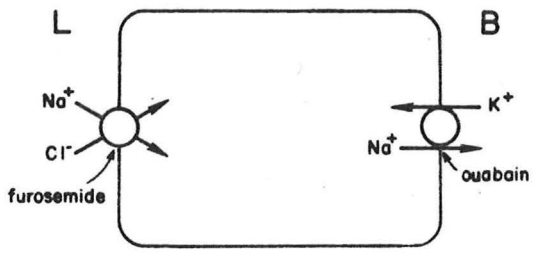

Figure 5. Schematic illustration of a distal tubule cell exhibiting electroneutral apical NaCl cotransport.

excellent recent evidence that lower concentrations of thiazides and metolazone inhibit this electrically silent apical transport process (36-39). It should be noted that this electroneutral NaCl cotransporter is a different transport system than the electroneutral entry of NaCl via parallel Na-H and Cl-HCO_3 exchange. This particular transport process has been difficult to directly examine in the kidney because of its rather limited location to the distal tubule. However, recent studies are beginning to characterize the distal tubular cell and have directly confirmed the presence of a thiazide sensitive NaCl uptake mechanism (40,41).

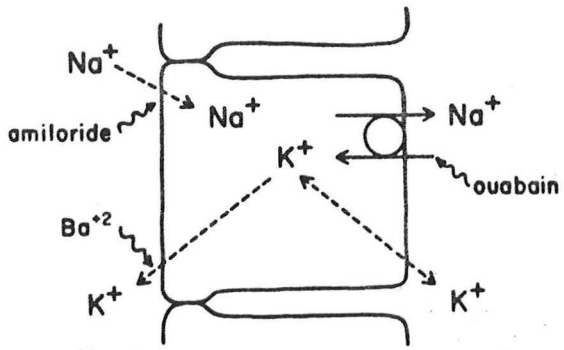

Figure 6. Schematic illustration of the principle cell in cortical collecting tubule. See text for details.

The final cell type of importance with respect to diuretics exists in the collecting duct system and is characterized by the principle cell. A model of this cell type is shown in Figure 6. Na absorption by the principle cell of the collecting tubule is active and generates a lumen negative voltage. Na enters the cell across the apical cell membrane via a conductive channel that can be blocked by amiloride (42,43). Na exits the cell across the basolateral membrane via the NaK ATPase pump. The Na pump normally maintains a [K] in the cell above electrochemical equilibrium. Both apical and basolateral cell membranes of the principle cell have high K conductance with the apical membrane conductance being somewhat higher (42). Thus, the principle cell normally exhibits net potassium secretion. It is important to note that aldosterone stimulates potassium secretion and sodium absorption in this segment via several mechanisms that include increasing apical cell membrane Na conductance, increasing apical cell membrane K conductance, and increasing NaK ATPase activity (42, 44). It is in this cell type that the K sparing diuretics, amiloride, triamterene, and spironolactone have their effect. Amiloride inhibits Na absorption by blocking apical cell membrane Na conductance. Triamterene has a similar effect. Spironolactone inhibits Na absorption by competitive binding aldosterone for the aldosterone receptor. As a result, all the aldosterone sensitive transport mechanisms are blunted including apical Na and K conductances, and NaK ATPase activity.

III. Altered Extrarenal Transport in Hypertension--Role for Extrarenal Antihypertensive Effects of Diuretics

A number of recent hypotheses and observations have suggested an "electrolyte dependent" cause for hypertension. Blaustein has formu-

lated a hypothesis that hypertension is related to cation movements in vascular smooth muscle (45). This hypothesis suggests that intracellular calcium is the cause for sustained smooth muscle tension and increased peripheral resistance seen in hypertension. Elevated intracellular [Ca] is linked to an elevated intracellular Na which, via sodium calcium exchange, results in enhanced Ca entry into the cell. If this hypothesis is correct and can be generalized to additional cells in the body, then observations on the intracellular Na and Ca content in red blood cells may relate to the pathophysiological events in hypertension. A number of studies have demonstrated abnormal cation transport in red blood cells of patients with essential hypertension (46-53). While it is not the purpose of this presentation to define the pathophysiology of hypertension, Figure 7 illustrates the ratio of Na to K flux in red cells from normal and hypertensive patients. Patients with essential hypertension and normotensives with a family history of hypertension demonstrate a decreased ratio of Na to K flux in red cells in vitro (49). Shown in Table I are Na and K fluxes in red cells from normotensive and hypertensive white and black patients. These fluxes are furosemide sensitive (53). In blacks, furosemide-sensitive Na and K efflux rates are significantly lower tha nthose observed in red cells from whites. In addition, K efflux rate in hypertensive blacks is significantly lower than in normotensive blacks.

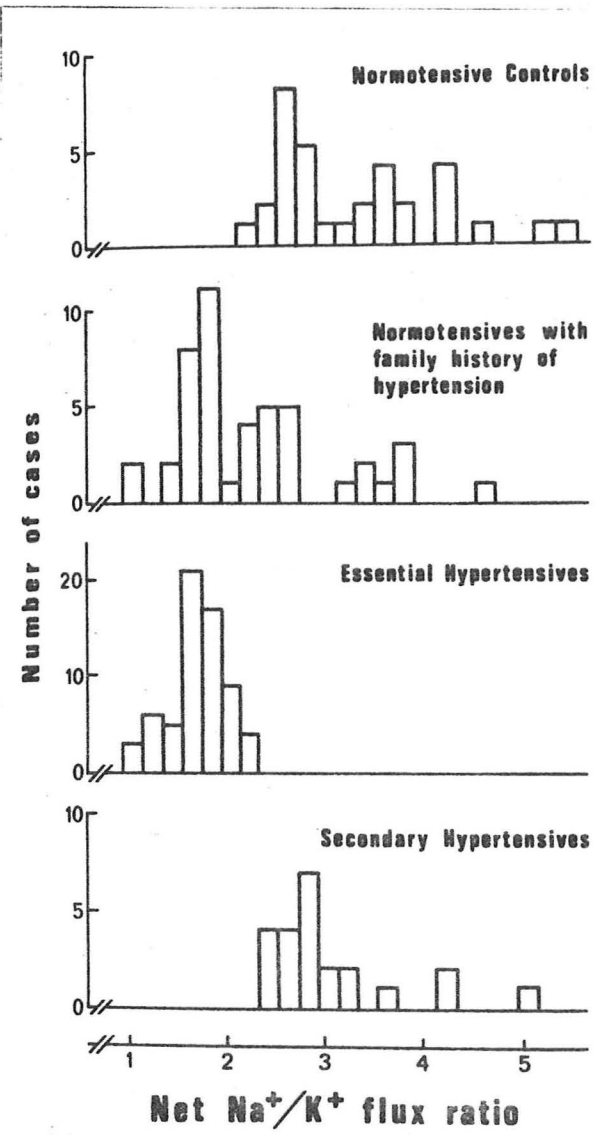

Figure 7. Histograms of the in vitro Na/K flux ratio in Na loaded K depleted red blood cells. Patients with essential hypertension and relatives of patients with a family history of hypertension exhibit increased Na/K flux ratio. From reference 47.

It is obvious that alterations in diuretic-sensitive transport systems in red cells per se probably do not play a role in hypertension. The possibility that the red cell is a marker for generalized abnormal transport process may explain why diuretic agents affect hypertension by hemodynamic changes that may be independent of the renal diuretic activity.

TABLE I

KINETIC PROPERTIES OF RED CELLS

	Furosemide-Sensitive Effluxes	
	Na	K^+
	μmol/L cells/hr	
White		
Normotensive	629	584
Hypertensive	608	612
Black		
Normotensive	264	320*
Hypertensive	256	265

*Significantly greater than hypertensive blacks. From reference 33.

Long-term diuretic therapy of essential hypertension is usually associated with a drop in peripheral resistance (54, 55). This observation has lead to a number of investigations into the mechanism whereby diuretics may effect vascular resistance. Figure 8 shows the effects of the loop diuretic piretanide which inhibits the Na-K-2Cl cotransporter on diastolic blood pressure in nephrectomized spontaneously hypertensive rats (56). Figure 9 demonstrates the effects of piretanide on coronary blood flow in isolated guinea pig hearts (56). Both of these experiments demonstrate a vasodilatory effect of this diuretic. Although a

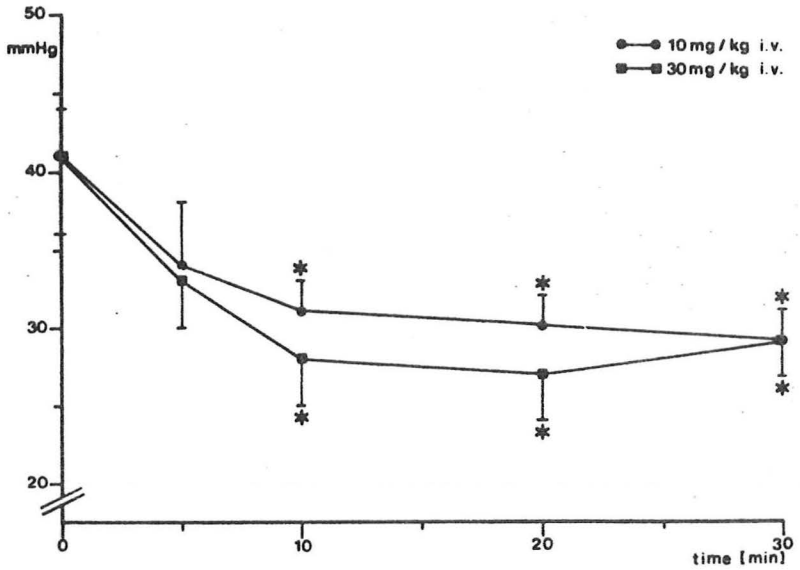

Figure 8. Effect of piretanide on diastolic blood pressure of bilaterally nephrectomized, pithed, spontaneously hypertensive rats. From reference 56.

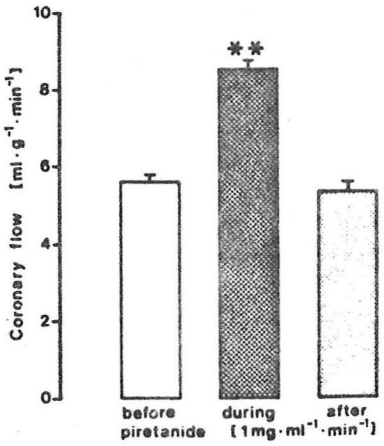

Figure 9. Effect of piretanide on coronary blood flow in isolated guinea pig hearts. From reference 56.

mechanism for these vascular effects is unclear, it may be related to the presence of furosemide-sensitive Cl transport in vascular smooth muscle (57). Several of the commonly used and new diuretics have also

been demonstrated to significantly reduce peripheral resistance or directly effect vascular smooth muscle contraction. Table II illustrates the concentrations of diuretics producing 50% inhibition of vascular smooth muscle contraction either spontaneously or under stimulation of angiotensin II or $PGF_{2\alpha}$.

TABLE II

Concentrations (μm) of diuretics producing 50% inhibition of spontaneous activity and Angtiotensin II-induced contractions in portal vein and $PGE_{2\alpha}$-induced contractions of aorta

Diuretic	Amplitude of Spontaneous Contractions	Ag-II Contractions	$PGF_{2\alpha}$ Contractions
Amiloride	3.4	6.8	18.5
Furosemide	4.5	76.2	68.3
Piretanide	5.5	83.8	
Bumetanide	0.6	82.6	
Hydrochlorothiazide	174.5	840.7	280.7
Indapamide	53.7	286.4	131.3

From reference 58

Even though it has been demonstrated that certain diuretics effect vascular resistance, it would be important to determine if the acute and chronic effects, i.e. the observed chronic effect of a decrease in peripheral resistance, are due to the same mechanism and whether they are due to effects of diuretics on ion transport in vascular smooth muscle as opposed to indirect effects via stimulation of renin and angiotensin as well as vaso-active arachidonic acid metabolites.

IV. New Diuretic Agents

When one reviews the currently available diuretics from the standpoint of ability to produce natriuresis and diuresis, there appears to be no need for new agents. Indeed, the commonly held feeling is that most diuretics which act at a specific nephron site are roughly equivalent. The most important reasons for not holding such a point of view is the fact that diuretic complications are many and continue to produce significant morbidity and mortality. Table III summarizes the most common complications associated with diuretic treatment. While this presentation cannot address in detail the mechanisms for each of these complications, in discussing some of the newer agents their advantages with respect to these complications will be pointed out.

TABLE III
COMPLICATIONS OF DIURETIC USE

1. Massive natriuresis → "pre-renal" azotemia → shock
2. Hypokalemia → sudden death
3. Hypomagnesemia → sudden death
4. Hyponatremia
5. Hyperuricemia
6. Carbohydrate intolerance
7. Hyperlipidemia
8. Metabolic alkalosis
9. Ototoxicity
10. Nephrotoxicity

3-sulfamoyal-4-chlorobenzoic acid hydrazides

Figure 10 shows the structure of this group of compounds which are closely related to other sulfamonides such as the thiazides. Without attempting to discriminate between these compounds, this discussion will center mostly on indapamide which entered the U.S. market as Lozol in August of 1983. In the pharmaceutical industry it has been popular to modify the sulfamonide molecule in an attempt to discover clinically significant differences in the treatment of hypertension. Such modification of the sulfamonide molecule has been extremely successful, producing a number of antibiotics, oral hypoglycemic agents, carbonic anhydrase inhibitors, thiazide diuretics, and the vasodilator diazoxide. A series of indoline and isoindoline derivatives of chlorosulphonamide were synthetized and tested in models to look for diuretic and antihypertensive action. Indapamide was shown to be a long-acting hypotensive with minimal diuretic activity. It has undergone clinical trials in over 6,000 patients worldwide and results from 26 control studies show that a single dose of 2.5 mg indapamide per day results in a significant reduction of systolic and diastolic blood pressure which is directly related to the initial blood pressure (59).

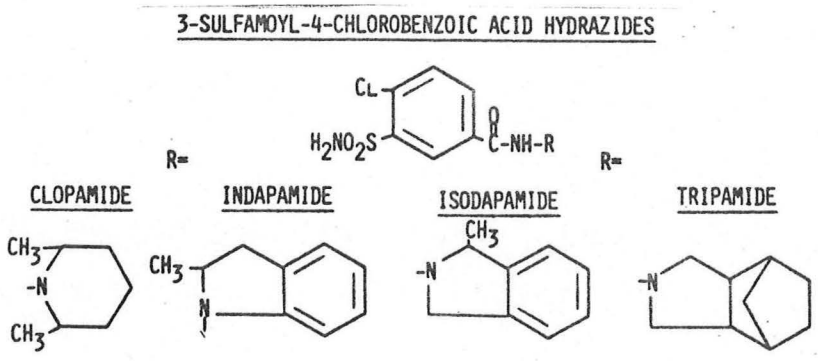

Figure 10.Structure of the 3-sulfamoyl-4-chlorobenzoic acid hydrazides.

Chemistry. Indapamide is a weak acid with a polar chlorosulphobenzamid substituent and a lipid soluble methyl indoline substituent. It is unstable in solution when exposed to light but in the dark at 4°C it is stable for at least two months. It has a relatively high pKa of 8.3 (60). The partition coefficient between octanol and phosphate buffer at pH 7.4 at 37°C is a measure of lipid solubility. Table IV illustrates this partition coefficient for indapamide and other diuretics. The very high partition coefficient for indapamide is responsible for its extensive uptake by red blood cells and other cells and its prolonged biologic half-life (60).

TABLE IV

PARTITION COEFFICIENTS BETWEEN OCTANOL AND PHOSPHATE BUFFER (pH 7.4)

Drug	Partition Coefficient
Indapamide	32
Chlorthalidone	5
Hydrochlorothiazide	1
Furosemide	0.4

Pharmacokinetics. Peak plasma concentration after an oral dose of indapamide in humans are reached between 1 and 2 hours. It is extensively taken up by red blood cells in a ratio of 4:1 to plasma (60). Absorption is relatively complete with 94% of the administered dose appearing in blood. Only 5% of the drug is excreted unchanged into the urine with most of the drug being metabolized by the liver yielding metabolites which are excreted approximately 60% in the urine and at

least 20% in the stool (60). The renal clearance of indapamide is approximately 5 ml/min which is probably a result of extensive tubular reabsorption of this highly lipid soluble compound. It has been postulated that this lipid solubility of indapamide results in proximal tubular reabsorption when low doses are administered. This results in distal delivery of very small quantities of the drug and thus minimal diuretic action (60). Indapamide is about 80% protein bound and has an apparent volume of distribution of 60 liters, again a reflection of its high lipid solubility.

Mechanism of Action. Stop flow studies in the dog kidney have demonstrated a distal tubular site of action (61). Based on the fact that indapamide along with metolazone produces increases in renin release only after a 1 hour delay as opposed to loop diuretics such as furosemide and bumetamide which cause an immediate rise in renal renin release, it has been postulated that indapamide has a site of action in the distal nephron distal to the thick ascending limb of Henle (62). Although one might anticipate that the cellular mechanism of action of indapamide involves apical cell membrane NaCl cotransport in the distal tubule, direct studies are not available.

Vascular Action. A number of investigators have demonstrated a direct vascular action for indapamide. Observations on the effects of indapamide on the amplitude of the action potential and contraction of vascular smooth muscle in the portal vein suggests that it may exert antihypertensive action by reducing the transmembrane calcium current (63, 64). Indapamide also inhibits the vascular response to norepinehprine and KCl in vitro, although this inhibitory effect is only mild (65).

Complications. It appears that indapamide in doses which produce significant diuresis, i.e. >2.5 mg results in similar types of complications observed with thiazides. However, when indapamide is given at its antihypertensive dose of 2.5 mg daily it appears to have significantly fewer and quantitatively less significant side effects. In the majority of clinical trials 2.5 to 5 mg per day of indapamide resulted in significant lowering of serum [K] but not below a mean of 3.5 mM (66). In patients with hypertension 2.5 mg per day of indapamide for up to ten months decreased estimated total body potassium insignificantly from 2,785 to 2,660 mM (67). This reduction is somewhat less than that seen with thiazide diuretic therapy of hypertension (68). A single dose administration of 2.5 mg of indapamide does not increase urinary magnesium excretion (69). However, higher doses definitely increase the rate of urinary magnesium excretion and reduce the serum magnesium concentration (70).

With respect to plasma uric acid concentrations, numerous studies have demonstrated that 2.5 mg of indapamide results in either no increase or a significantly smaller increase in serum uric acid compared to other diuretic therapy (71-73).

Glucose tolerance. There have been rare instances in patients who have had elevated blood sugar concentrations on indapamide therapy but almost none of the studies of carbohydrate metabolism in nondiabetic patients have demonstrated that indapamide alters glucose tolerance (74-76). In addition, in diabetic patients with hypertension, indapamide did not alter mean blood glucose concentration or insulin secretion for up to one year of therapy (77-79).

Lipid Metabolism. Long-term data are limited but administration of indapamide 1-4 mg daily to hypertensive patients for up to two years has not been associated with increases in total serum cholesterol or low density lipoprotein (76, 77).

Summary and Recommendation. As a diuretic to treat edema, indapamide appears to have no specific advantage over other currently available drugs. However, its apparent effect on vascular smooth muscle at weak diuretic doses suggests that this agent may be preferred in the initial management of hypertension. More long-term data from larger patient populations will be needed to confirm the apparent low side effect profile of indapamide.

Indacrinone

Ethacrynic acid is a member of the aryloxy aceticacid family. A number of additional compounds from this family have been synthesized. Figure 11 illustrates several additional members of this group of diuretics. It will be recalled that one of these agents, teinilicacid had been previously introduced as a uricosuric diuretic but removed from the market due to a significant hepatic toxicity (80). Indacrinone has been shown in micropuncture studies to have its principle site of action in the thick ascending limb of Henle as well as the early distal tubule (81). Presumably its mechanism of action mimics that of ethacrynic acid. As shown in Figure 12, indacrinone exists as two enantiomers. These enantiomers have different degrees of action in loop of Henle and distal tubule. Table V from reference 81 illustrates the responses of rat loop of Henle and distal tubule to the two different enantiomers (81).

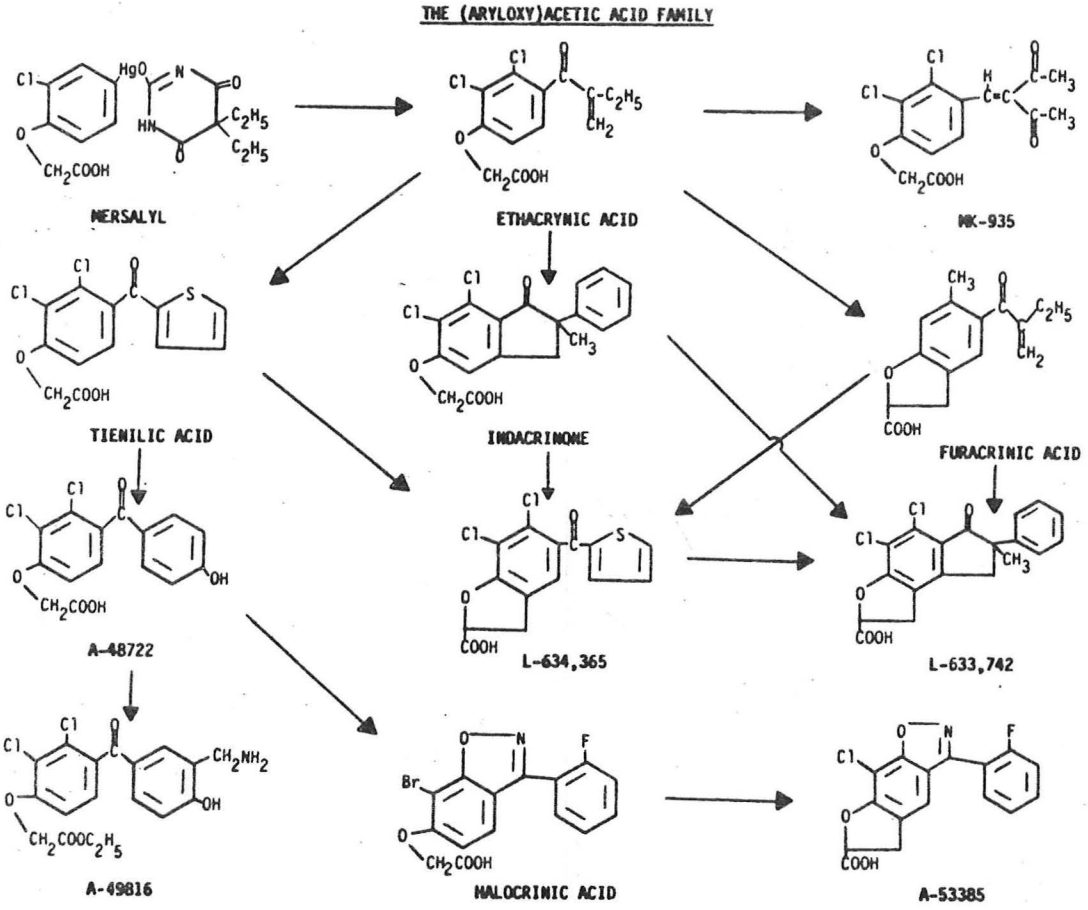

Figure 11 Structure of the aryloxyacetic acid family.

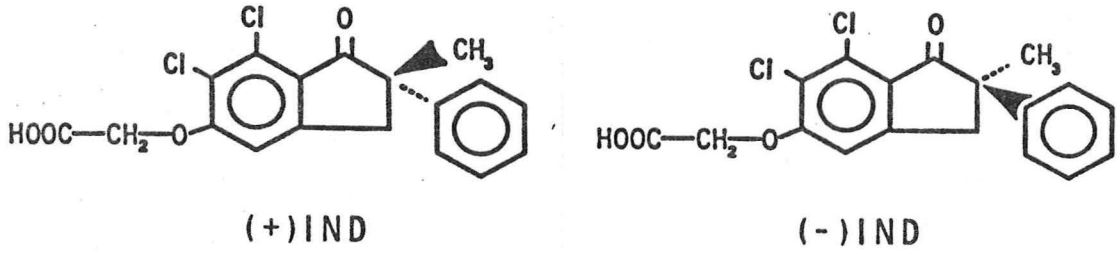

Figure 12 Enantiomers of Indacrinone.

TABLE V

EFFECTS OF ENANTIOMERS OF INDACRINONE

ON IN VIVO TUBULAR TRANSPORT

	Control	(-) Indacrinone	(+) Indacrinone
Loop of Henle			
Na	2270	930*	1620*
K	54.7	-2.1*	33.3*
Distal Tubule			
Na	369	159	209
K	-62.9	-55.2	-59.5

*p <0.05 vs control. All units are $pmol_{\circ}min^{-1}$. "+" absorption "-" secretion. From reference 81

It is evident that both enantiomers inhibit sodium absorption and potassium absorption in the loop of Henle while both also inhibit sodium absorption in distal tubule without effecting potassium secretion. The negative enantiomer was significantly more potent than the positive enantiomer. It should be noted that species differences appear to exist with respect to the relative potencies of the positive and negative enantiomer.

In studies in humans, indacrinone had a greater natriuretic potency and a longer duration of action than furosemide while inducing less potassium loss for any degree of natriuresis (82). During the period of maximum diuresis and natriuresis, indacrinone also produced uricosuria

(82). In patients with congestive heart failure, indacrinone induced diuresis has been shown to result in no clinically significant elevation of uric acid or decrease in serum potassium (83).

In normal human subjects, as shown in Figure 13, only the negative enantiomer resulted in significant increase in urinary sodium excretion. Similarly, in Figure 14 is illustrated the fact that the positive enantiomer resulted in significant reduction in serum uric acid concentration while the negative enantiomer resulted in a significant increase. Manipulation of the enantiomeric ratio with more positive than negative enantiomer has been shown to produce a diuretic with significant uricosuric properties (84).

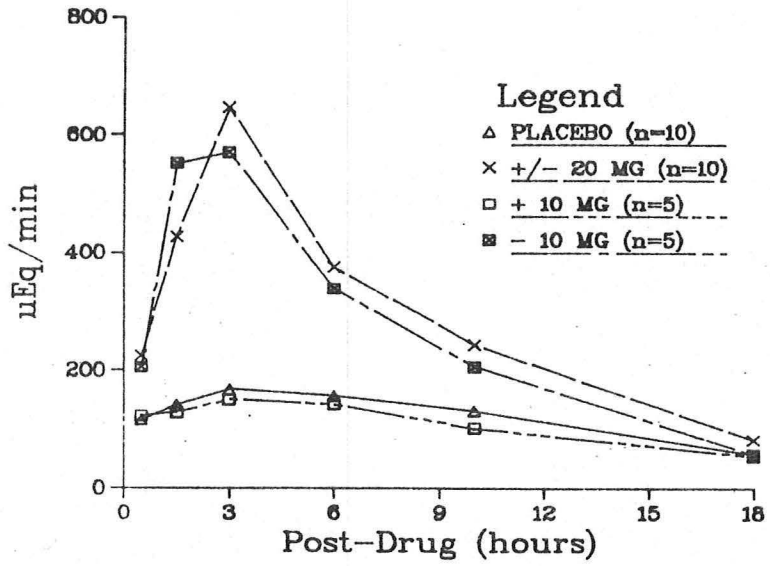

Figure 13 Time course of urinary sodium excretion after single doses of the enantiomers of indacrinone.

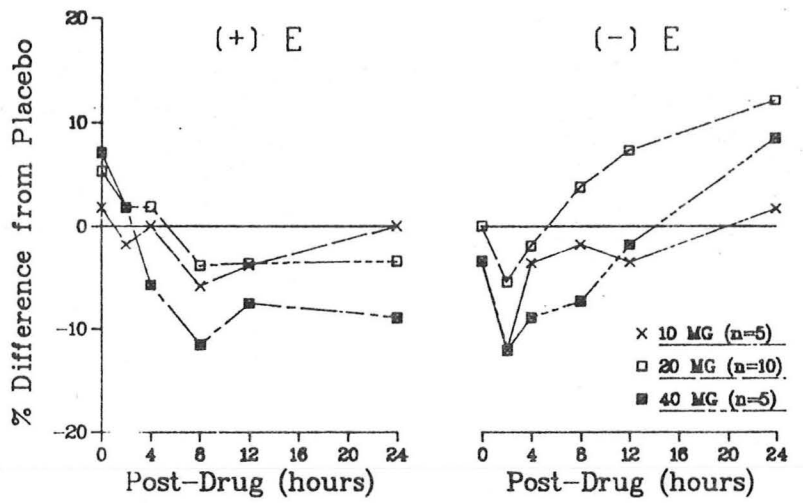

Figure 14 Dose response of mean serum uric acid concentration change produced by the enantiomers of indacrinone.

Muzolimine

The last new diuretic to be discussed is muzolimine which is a structurally novel loop diuretic presently under clinical evaluation. Musolimine has a natriuretic effect that lasts somewhat longer than that of the classical loop diuretics (85-87). This longer duration of action is related to different pharmacokinetic properties. Peak plasma concentrations after a single oral dose are observed one to three hours after administration. The biological half-life of muzolimine is approximately 12 to 16 hours (88). Thus, muozlimine is eliminated chiefly by extrarenal mechanisms.

The apparent clinical advantage of muozlimine lies in the fact that it is more potent than furosemide as a diuretic in the setting of renal failure (89-91). In addition its longer duration of action allows for once a day therapy in the treatment of hypertension or edema associated with renal failure, heart failure, or significant liver disease.

TABLE VI

EFFECTS OF DIURETICS ON ION EXCRETION

N° of cases	Drug and dose (mg)	% change in 24-h renal output				
		Cl	Na	Volume	K	Mg
13	amiloride 5	10^{ns}	29^{b}	19^{a}	-27^{c}	-4^{ns}
13	amiloride 10	32^{d}	65^{e}	43^{e}	-32^{d}	-7^{ns}
9	chlorthalidone 100	152^{e}	165^{e}	69^{d}	69^{d}	87^{d}
9	furosemide 40	49^{b}	48^{d}	49^{d}	21^{a}	52^{a}
19	hydrochlorothiazide 50	63^{e}	61^{e}	38^{e}	31^{a}	24^{a}
9	hydrochlorothiazide 50	59^{a}	63^{d}	35^{a}	22^{e}	44^{a}
7	indapamide 2.5	87^{c}	139^{e}	26^{c}	23^{e}	-8^{ns}
10	muzolimine 30	38^{e}	34^{c}	14^{c}	20^{ns}	8^{ns}
13	xipamide 5	39^{e}	46^{c}	20^{e}	40^{a}	27^{c}
13	xipamide 10	106^{e}	120^{e}	63^{e}	55^{a}	50^{e}
13	xipamide 20	101^{e}	111^{e}	63^{e}	79^{e}	40^{e}

Significances of the differences between mean excretions after drug and after placebo: $^{a}P < 0.05$; $^{b}P < 0.02$; $^{c}P < 0.01$; $^{d}P < 0.005$; $^{e}P < 0.001$; ns: non-significant.

With respect to side effects, there is no evidence that muozlimine at equipotent doses to furosemide has significantly less side effects with the possible exception of magnesium depletion. Table VI illustrates the percentage change in urinary magnesium excretion in response to a number of different diuretics. As can be seen, muzolimine in addition to indapamide and amiloride are the only diuretics which do not result in a significant increase in urinary magnesium excretion. This lack of magnesium wasting combined with a more sustained action of muzolimine may offer advantages above the rapid acting magnesuric agents such as furosemide.

Aquaretic agents

The final agents to review constitute a new class of diuretic agents which primarily increase renal water excretion. These agents are antagonists of the antidiuretic effect of arginine vasopressin and promise to have application in the treatment of various pathological states of water retention. The agent $d(CH_2)_5Tyr(Et)VAVP$ blocks the antidiuretic effect of arginine vasopressin, Figure 15 (92). This antagonistic effect of vasopressin analogs is related to the ability of the analogs to compete with vasopressin for binding sites, i.e. vasopressin receptors. Figure 16 illustrates the correlation between the affinity for vasopressin binding sites and the antagonistic potency for adenylate cyclase activation of 35 different vasopressin antagonists (93). Additional in vivo studies in the hydropenic rat demonstrate major reduction in urinary osmolality as a function of a dose of $d(CH_2)_5Tyr(Et)VAVP$ (SKF 100398), Figure 17 (94). While these agents remain to be applied in the clinical situation, they have great potential therapeutic utility in the treatment of syndromes of inappropriate ADH secretion. Also, these agents may be useful in the treatment of severe hyponatremia associated with excessive vasopressin secretion or inappropriately low free water clearance, i.e. postoperatively and during congestive heart failure.

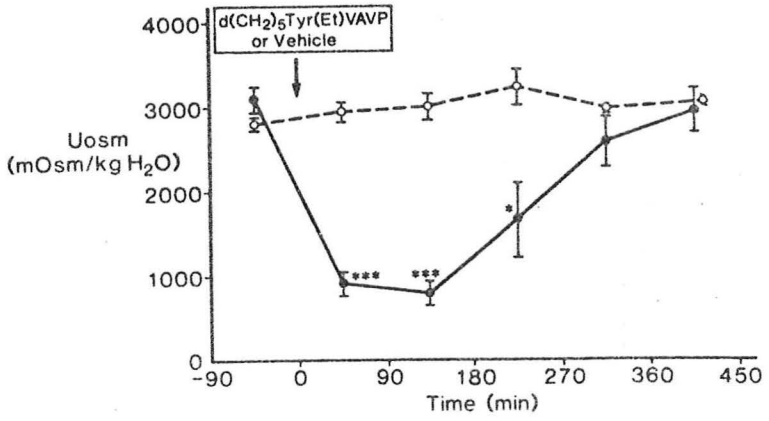

Figure 15 The response of urinary osmolality to $d(CH_2)_5Tyr(Et)VAVP$ in water-deprived rats. From reference 92.

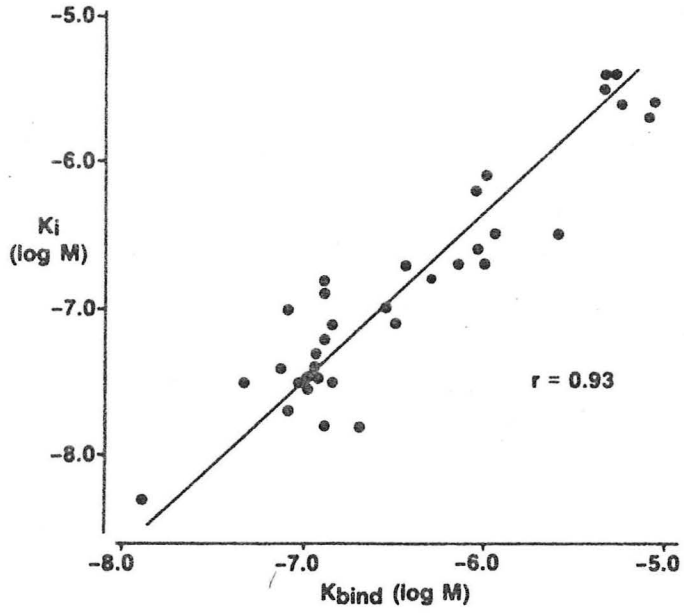

Figure 16 Correlation between adenylate cyclase inhibition and affinity for vasopressin binding sites of 35 vasopressin antagonists. From reference 93.

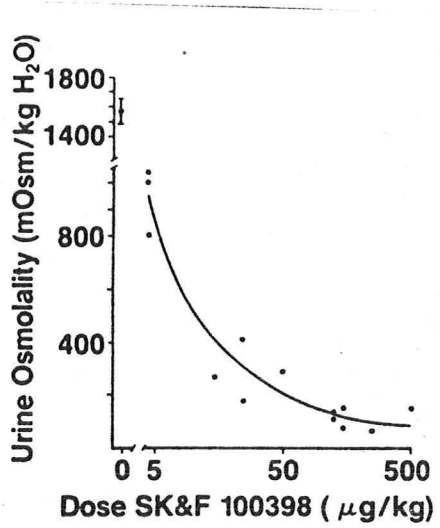

Figure 17 Dose-response of urine dilution to SKF 100398--a vasopressin antagonist. From reference 94.

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