INHIBITORS OF ION TRANSPORT AS THERAPEUTIC AGENTS: RECENT ADVANCES

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Internal Medicine Grand Rounds

August 18, 1988

INTRODUCTION

Enzyme-specific inhibitors have historically had tremendous utility as therapeutic agents. Often the clinical benefits of such agents were appreciated long before the pharmacologic action of the given drug was delineated. Recently, spectacular advances in the development of therapeutic agents have accelerated with the resolution and characterization of target enzymes. Examples include the synthesis of angiotensin converting enzyme (ACE) inhibitors (1), and the development of compactin (2), made possible by the definition and purification of HMG CoA reductase, the rate-limiting step in cholesterol synthesis (3,4).

Yet another broad class of therapeutic agents have derived from advancing knowledge of target-tissue specific cell membrane and intracellular hormone receptors. Here there has been tremendous cross-fertilization of clinical pharmacology and basic research. Physiologic identification of the gastric mucosa histamine H_2 receptor (5,6) led to the evolution of histamine H_2 receptor antagonists (e.g., cimetidine) (7) as therapeutic anti-ulcer agents and, conversely, the well-known central nervous system effects of opiates led to the identification of the opiate receptors of brain (8). It can be expected that definition of the latter will, in turn, advance the development of newer, modified opiate receptor agonists and antagonists.

In addition to inhibitors of classic enzyme systems and ligand-specific receptors serving as therapeutic agents, considerable development of plasma membrane transport inhibitors has occurred over the past 50 years. Perhaps the most widely used group of such agents are diuretics, with highly specific inhibitors such as bumetanide (Bumex) and amiloride seeing daily hospital usage (9). Recent developments in this class of therapeutic agents were reviewed in this forum in 1985 (10).

In this monograph, I would like to review recent advances in yet another group of inhibitors of ion transport, those which interfere with ATP-driven ion pumps. As is the case with therapeutic agents in general, some inhibitors of ion transporting ATPases have been in longstanding clinical use before their mechanisms of action have been defined. The mechanism of action of two such compounds, digoxin and chloroquine, have recently undergone elucidation owing to a fuller description of the affected transporter, and in this Grand Rounds, these advances will be reviewed. In addition, the potential therapeutic benefits of a new type of ATPase inhibitor, omeprazole, will be reviewed as an example of a system in which our understanding of the ion transporter itself has led to ongoing investigation of useful clinical agents.

CLASSES OF ION TRANSLOCATING ATPases

As distinguished from most molecular mechanisms of ion transport, such as the renal sodium-proton antiporter (11) and the cardiac sodium-calcium exchanger (12), ion translocating ATPases are directly energized. That is, the energy of the terminal phosphate bond of ATP is cleaved by the ATPase and, through a variety of energy transducing mechanisms, this energy is used to drive ion movement through the protein and across a membrane to create an ionic gradient. A mechanistic comparison of secondarily active and primary active transport is shown in Figure 1.

Ion translocating ATPases of mammalian systems can be placed in three major classes, as shown in Table I. First is the F_1F_o type ATPase, represented by the mitochondrial ATP synthetase. As indicated, this is a large (≧350 kDa) heteroligomer compound of at least 9 subunits. This enzyme operates as the terminal step in oxidative phosphorylation and utilizes the chemical and osmotic energy of the mitochondrial proton gradient established

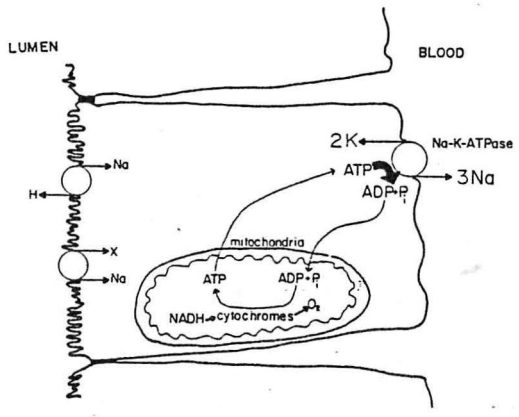

Figure 1: Comparison of Primary and Secondarily Active Transport. The primary active, ATP energized, Na^+K^+ ATPase drives sodium from the cell, creating a low intracellular [Na] relative to lumen and blood. Na^+ enters the cell across the apical membrane, an event energized by the established Na^+ gradient and, because of fixed coupling, H^+ exits the cell against an H^+ gradient, as an example of secondarily active transport.

TABLE I

Classes of Ion Translocating ATPases

Class		Examples	Molecular Mass	Subunits
I.	F_1F_o	mitochondrial ATPase/synthetase	350	9
II.	E_1E_2	Na^+-K^+ ATPase	165	2 (α,β)
		H^+-K^+ ATPase	92	1
		Ca^{2+} ATPases	~100	1
III.	Endomembrane or vacuolar	proton pumps of clathrin-coated vesicles, endosomes, lysosomes, golgi, endoplasmic reticulum, chromaffin granules, synaptic vesicles, tertiary granules of leukocytes	~530	8-9

by the electron transport chain to fuel the synthesis of ATP from ADP and P_i (13), as shown in Figure 2.

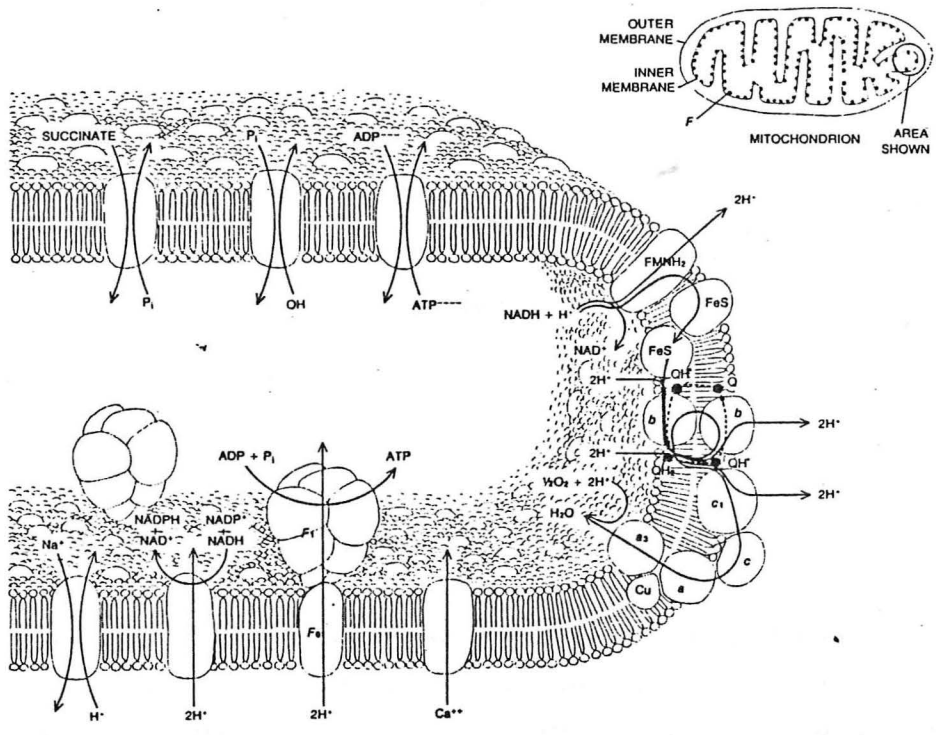

Figure 2: F_1F_0 ATPase: The Mitochondrial ATP Synthase. Illustrated is the electron transport chain and ATPase of the inner mitochondrial membrane.

Those pumps of the second class are referred to as E_1E_2 type ion translocating ATPases, and include the Na^+-K^+ ATPase, the Ca^{2+} ATPases and the H^+-K^+ ATPase of gastric mucosa. The designation "E_1E_2" refers to the nature of the energy transducing step in which the energy of ATP hydrolysis is coupled to ionic movement (14). Specifically, the terminal phosphate of ATP is directly transferred to the enzyme, to form a phosphoaspartyl intermediate,

and this phosphorylation event induces a conformational change in protein resulting in the directional movement of the transported ion as shown in Figure 3. As listed in Table I, these types of ion translocating ATPases are much simpler than F_1F_o type pumps, and are composed of one to two subunits with a total molecular mass of 90-165 kDa.

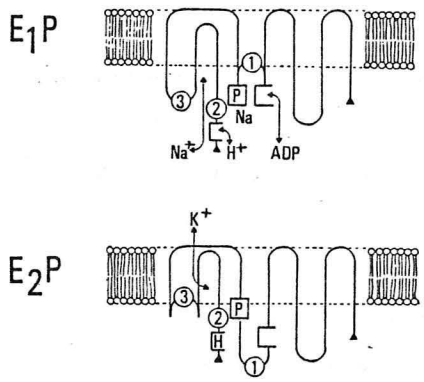

Figure 3: Catalytic Cycle of the E_1E_2 Type ATPase, NaK ATPase. Direct phosphorylation of the enzyme by ATP accounts for conformational changes, designated E_1 and E_2 and is responsible for the sequential ionic movement of Na^+ and K^+.

The third type of ion translocating ATPases is the newest class of ion pumps and function exclusively to transport hydrogen ions, or protons, to create pH gradients. These are also large, heteroligomers and biochemically, they are distinguished from F_1F_o type ATPases not only by differences in inhibitor sensitivies, but also in localization (they are exclusively non-mitochondrial) and by the physiologic distinction that they hydrolyze ATP to move protons and create a pH gradient, whereas the mitochondrial proton pump utilizes an existing proton gradient to synthesize ATP (15). A working model of an endomembrane proton pump is shown in Figure 4.

Having reviewed the general classes of ion translocating ATPases, specific examples of inhibitor-pump interactions will be discussed.

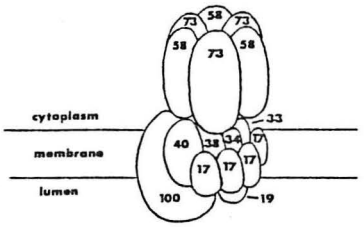

Figure 4: The Clathrin-Coated Vesicle Proton Pump, an Endomembrane Type Proton Translocating ATPase.

THE SODIUM POTASSIUM ATPase: THE MOLECULAR BASIS OF TISSUE SPECIFICITY IN CARDIAC GLYCOSIDE ACTION

Cardiac glycosides (clinically, digoxin; experimentally, ouabain) have been used for the treatment of cardiac arrhythmias and congestive heart failure for at least 200 years. Understanding of the action of cardiac glycosides has proceded in two fronts with the physiologic definition of ion trafficking events in the polarization of excitable and non-excitable tissues, and with the definition of the ouabain target site, Na^+-K^+ ATPase (16,17).

Sodium-potassium ATPase subserves a wide variety of cellular functions, including regulation of cell volume, maintenance of excitability in nerve and muscle, regulation of salt and water retention by the kidney, control of glycolysis and respiration, and maintenance of an appropriate environment (high intracellular K^+ concentration) for protein synthesis.

Structurally, Na^+K^+ ATPase is an integral membrane protein comprised of two different subunits, α and β, present in a 1:1 ratio. The α subunit is the catalytic subunit, and contains both ATP and ouabain binding sites. The function of the β subunit remains unknown. Functionally, the enzyme serves to move Na from the cell and K into the cell and operates with a stoichiometry of 1 mole ATP:3 moles Na^+:2 moles K^+ (18). A schematic view of Na^+-K^+ ATPase is shown in Figure 5.

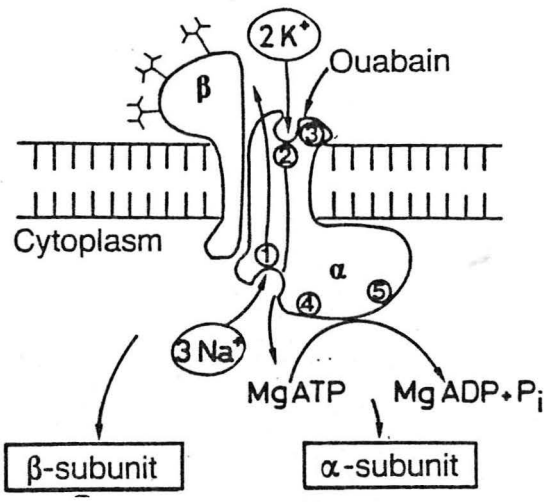

Figure 5: Schematic Model of Na^+K^+ ATPase.

The inhibitory effect of cardiac glycosides on Na^+K^+ ATPase are well described (19). The positive ionotropic effect of ouabain and digoxin on cardiac contractility are explained by a secondary effect resulting from partial inhibition of the enzyme. Cardiac Na^+K^+ ATPase operates in parallel with a secondarily active Na^+/Ca^{2+} exchanger, and the latter serves as a fine regulator of intracellular calcium activity in cardiac myocytes. With partial inhibition of the Na^+K^+ ATPase by cardiac glycosides, a slight elevation in intracellular Na^+ is observed, and this in turn, because of obligate coupling of Na^+ and Ca^{2+} movement through the Na^+/Ca^{2+} exchanger, leads to a rise in intracellular Ca^{2+}. This mechanism is depicted schematically in Figure 6.

The effects of intracellular Ca^{2+} on enhancing the force of muscle contraction relate specifically to regulation of troponin-tropomyosin interaction and activation of Ca^{2+}-dependent myosin light chain kinase (20). Thus, from a physiologic standpoint, the mechanism of digoxin's action on the heart is well described.

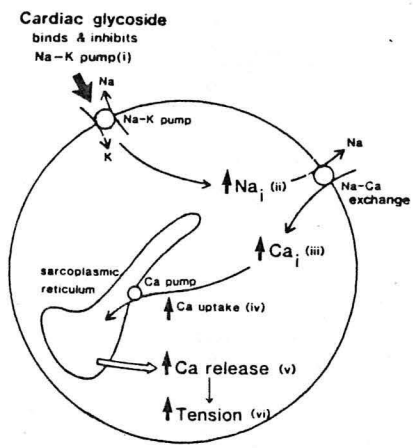

Figure 6: Effects of Cardiac Glycosides on Cardiac Muscle Cell. Diagram of a cardiac muscle cell showing the conventional view of the mechanisms underlying the positive inotropic actions of cardiotonic steroids (the hypothesis of Na^+-K^+-pump inhibition and Na^+/Ca^{2+} exchange). These drugs bind to and inhibit the Na^+-K^+ pump (i), thereby elevating the intracellular Na^+ concentration (ii); this increases the cytosolic free Ca^{2+} concentration, Ca (iii). In turn, this leads to an increase in the Ca^{2+} content of the SR (iv), so that Ca^{2+} release from the SR (v) and tension (vi) are increased when the muscle is subsequently activated.

What has not been reconciled in the description of ouabain's action is its tissue specificity. The kidney is an extraordinarily rich source of Na^+K^+ ATPase, and it is well known that renal sodium reabsorption is dependent upon Na^+K^+ ATPase, yet digoxin is seldom prescribed as a diuretic. Theoretically, it should be enormously potent. Na^+ is reabsorbed predominantly in three segments of the kidney, the proximal tubule (21), the thick ascending limb of Henle (22) and the cortical collecting duct (23). Although the luminal entry step for Na^+ is different in these three segments, each segment has basolateral membrane Na^+K^+ ATPase which serves not only to maintain a low intracellular $[Na^+]$, and hence a driving force for luminal Na^+ entry, but also to actively extrude reabsorbed Na^+ across the basolateral membrane and hence

effect net transepithelial Na^+ movement (24). A schematized view of this process is shown in Figure 7.

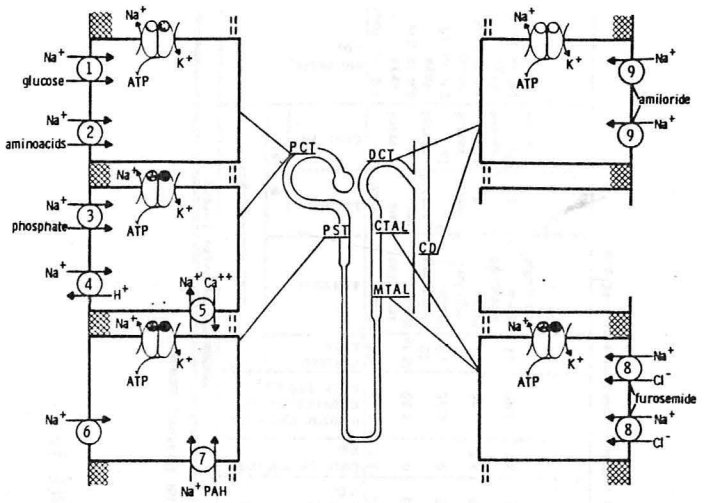

Figure 7: Survey of Various Modes of Renal Transtubular Na^+ Transport.

The ineffectiveness of digoxin in evoking a direct diuretic effect is supported by studies conducted by Kellum (25), in which normal human subjects were orally dosed with digitalis sufficient to achieve a "therapeutic" plasma level as assessed by EKG changes, including PR prolongation. As shown in Table II, digitalis administration under these conditions had no effect of urinary Na excretion.

Under different conditions, however, cardiac glycosides can have a direct natriuretic effect in the kidney. It has been demonstrated that digoxin, when directly infused into the renal artery of dogs, produces a prompt natriuresis (26). Moreover, studies conducted with isolated perfused proximal tubules (21), thick ascending limb (22) and cortical collecting duct (23) have demonstrated, in vitro, that ouabain can obliterate Na^+ reabsorption in these

TABLE II

SUMMARY OF ACTION OF DIGITALIS ON HEALTHY SUBJECTS

SUBJECT	CONTROL PERIOD								PERIOD OF DIGITALIS EFFECT								
	PULSE, BEATS EACH MINUTE	AVERAGE DAILY OUTPUT OF URINE, C.C.	LOSS OF WEIGHT, KG.	SODIUM CHLORIDE IN URINE, GM. IN EACH 100 C.C.	ELECTROCARDIOGRAM				PULSE, BEATS EACH MINUTE*	AVERAGE DAILY OUTPUT OF URINE, C.C.	LOSS OF WEIGHT, KG.	SODIUM CHLORIDE IN URINE, GM. IN EACH 100 C.C.	ELECTROCARDIOGRAM				DIGITALIS, GM.
					CARDIAC RATE	RHYTHM	T-WAVE (LEAD III)						CARDIAC RATE	RHYTHM	T-WAVE		
															LEAD II	LEAD III	
1 (experiment 1)	74	625	1.9	0.22	86	Sinus regular	Inverted		53	635	1.1	0.10	56	Sinus irregular	Diphasic	Inverted	1.6 in four days
1 (experiment 2)		700	0.3	0.47	90	Sinus regular	Inverted			790	0	0.63	56 to 72	Sinus irregular	Inverted	Inverted	2.0 in five days
1 (experiment 3)	88	677	0.6	0.20	93	Sinus regular	Inverted		68	755	0	0.15	68	Sinus regular	Inverted	Inverted	2.0 in 48 hours
2 (experiment 4)	71	475	0.9	0.39	60	Sinus regular	†		58	478	3.7	0.28	51	Sinus irregular	†	Inverted	1.6 in four days
3 (experiment 5)	66	637	0	0.12	72	Sinus irregular; ventricular premature contraction	†		58	562	0.9	0.09	67	Sinus irregular	†	Unknown	1.6 in four days

*Pulse rate taken when patient was under basal metabolic conditions at 7:30 A.M.

Subjects were maintained on 1.5 g NaCl/day diets, excepting subject 1 in experiment 2, who received 3.0 g NaCl/day. Both control and experimental periods consisted of 4 days.

segments. It is notable, however, that much higher concentrations of ouabain (10^{-5} M) were used in these experiments than is achieved during therapeutic digoxin administration.

From the foregoing, it would seem that differences in digoxin's effects on the heart and the kidney might owe to two possible explanations. First, as discussed above, digoxin's effect on cardiac contractility occurs because of the minimal elevation in intracellular Na^+ which develops after partial Na^+K^+ ATPase inhibition, with the resultant effect of increasing intracellular Ca^{2+}. Thus, it was considered a possibility that the specificity of digoxin's effect on the heart owed to the existence of a unique, parallel transporter in that organ - the Na^+/Ca^{2+} exchanger (12). This idea fell from credence when it was discovered that an almost identical system exists in the proximal convoluted tubule and in the distal nephron (27). In this setting, modest inhibition of the Na^+K^+ ATPase (or excessive Na entry into the cell) results in an increase in intracellular Ca^{2+}, and Ca^{2+} in turn completes a feedback loop by inhibiting apical Na^+ entry, as shown in Figure 8.

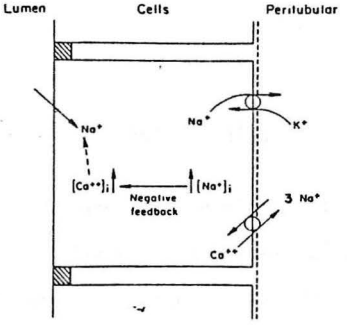

Figure 8: Relationship of Na^+-K^+ ATPase and the Na^+/Ca^{2+} Exchanger in the Nephron.

A second explanation for the tissue specificity of digoxin resides in the noted difference in the concentrations of cardiac glycosides used in oral loading of patients as opposed to the direct renal artery injections and in vitro studies. The first direct evidence for tissue differences in the sensitivity of Na^+K^+ ATPase to cardioglycosides evolved in 1979 (28). To date, at least three isozymes of the catalytic subunit of Na^+K^+ ATPase have been identified, two as proteins (29) and a third as a cDNA and mRNA (30). In all instances, the isoforms of the enzyme owe to intrinsic differences in the α chain, and the three forms are referred to as $\alpha 1$ (formerly α), $\alpha 2$ (formerly $\alpha(+)$) and $\alpha 3$ (31). Figure 9 illustrates the tissue specific expression of these isoforms. As shown renal Na^+K^+ ATPase is almost exclusively $\alpha 1$, whereas cardiac tissue contains both $\alpha 1$ and $\alpha 2$.

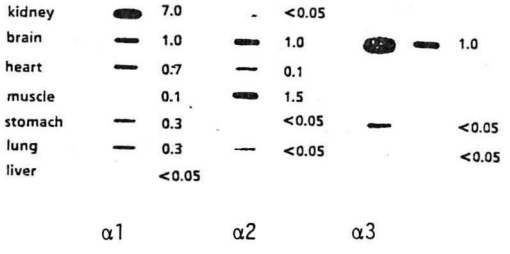

Figure 9: Relative Levels of mRNA Encoding the α Isoforms of Na^+K^+ ATPase in Various Tissues.

In addition, as shown in Figure 10, the $\alpha 2$ form of the enzyme within the heart is almost exclusively localized to the ventricles. This is consistent with the demonstration that there are two classes of ouabain binding sites in the heart, with the higher affinity binding site being localized to the ventricles (32).

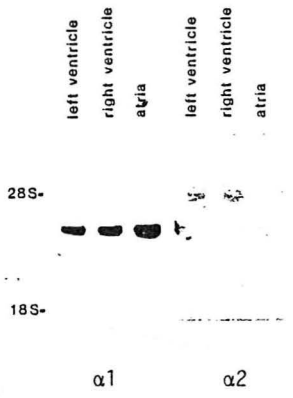

Figure 10: Relative Levels of mRNA Encoding the α Isoforms of Na^+K^+ ATPase Present in Rat Atrium and Ventricle.

Shown in Figure 11 is the sensitivity to ouabain of the α1 form, prepared from kidney and the α2 form, prepared from axolemma (33). As indicated, there is a dramatic difference in the ouabain sensitivity between the two isoforms with the kidney form (α1) being approximately 100-fold less sensitive to ouabain than the α2 form, of axolemma (and heart). To date, the α3 form has not been isolated biochemically and its relative ouabain sensitivity has not been characterized.

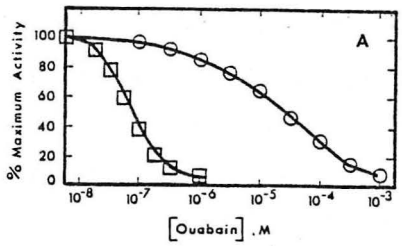

Figure 11: Sensitivity of α Isoforms of Rat Na^+K^+ ATPase to Ouabain. Circles represent kidney (α1) and squares axolemma (α2).

In addition to endogenous differences in ouabain sensitivity, the α1 and α2 forms of the enzyme are distinct with respect to Na affinity (34), and regulation by insulin and thyroid hormone (35).

With the cloning and sequencing of the cDNA encoding the α1, α2, and α3 forms (30), a tentative secondary structural model has been proposed in which the α subunit is composed of 7 to 8 transmembranes, α helical segments as shown in Figure 12 (36).

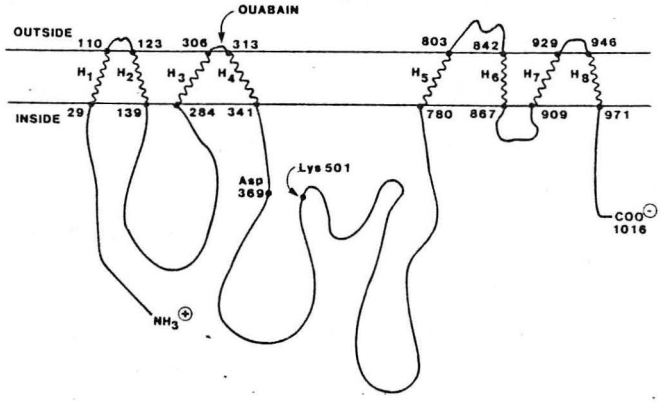

Figure 12: Model of Na^+K^+ ATPase α Chain Based upon cDNA Sequence. In addition, the ouabain binding site has been tentatively identified, and as shown in Table III, the primary amino acid sequence of the various isoforms differ in this region (30,36). In further confirmation that the α1, or ouabain-resistant form, is the predominant form present in the nephron, recent studies have been performed with anti-peptide antibodies prepared against peptides synthesized from α1-specific regions. By immunocytochemical analysis, dense basolateral membrane labelling was seen with anti α1-specific antibodies in the proximal tubule, thick ascending limb of Henle and cortical collecting duct (37). However, staining with an anti α2 antibody has not been

TABLE III

Predicted Amino Acid Sequence of Probable Ouabain Binding Site

α1		glu-tyr-thr-trp-leu-glu	
α2	H3 ←	gly-tyr-ser-trp-leu-glu	→ H4
α3		gly-tyr-thr-trp-leu-glu	

performed, and limited biochemical analysis based upon ouabain sensitivity and binding indicates that the α2 form of the enzyme may also be present in the cortical collecting duct (38).

Nonetheless, tissue specificity of cardiac glycoside action can largely be explained by differences in distribution of isoforms of Na^+K^+ ATPase which vary in their sensitivities to ouabain. It should be reemphasized that these isoforms differ only in relative sensitivity to cardiac glycosides, and that with either intrarenal administration of digoxin, or severe overdosage, inhibition of Na^+K^+ ATPase in relatively resistant tissues can occur. Indeed, it has been shown that the hyperkalemia associated with profound digoxin intoxication owes to potassium leakage from muscle, a tissue comprised of both α1 and α2 types of Na^+K^+ ATPase (39).

Finally, with the identification of isoform specific domains in α1, α2 and α3 Na^+K^+ ATPase, the opportunity arises for developing and screening inhibitors of the enzyme which are specific for ouabain-resistant isoforms. Such an inhibitor would be of enormous benefit in achieving a natriuresis in patients which are resistant to diuretic agents.

CHLOROQUINE: CONVERGENCE OF CLINICAL AND EXPERIMENTAL OBSERVATIONS

Chloroquine is the most important prophylactic agent in the prevention of malaria. Despite the fact that it has been in use for over 40 years as a

therapeutic agent for malaria (and more recently, as a second line agent for the treatment of systemic lupus erythematosis and rheumatoid arthritis), until recently, its mode of action has been ill (and mis-) understood (40,41).

In order to explain the mechanism of chloroquine action, it is first necessary to review important characteristics of acidic intracellular compartments. As noted previously, the newest class of ion translocating ATPases are proton pumps which are extramitochondrial in location. Indeed, these proton pumps have been localized to almost all intracellular compartments, excepting the nucleus, mitochondria and mature insulin secreting granules (15). Shown in Figure 13 is a schematic representation of the cell depicting the acidity of intracellular compartments.

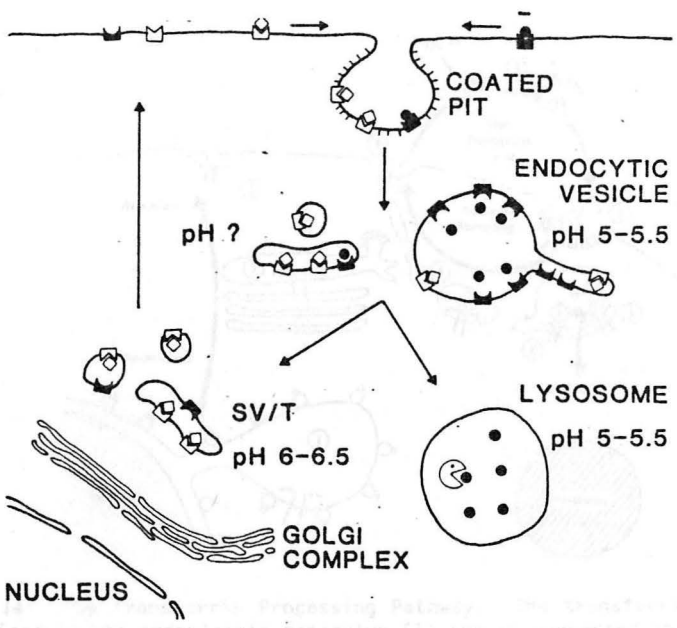

Figure 13: pH Profile of Endomembranes.

As indicated, there is an interorganelle pH gradient which serves to facilitate the processing of endocytosed receptor ligand complexes as well as various toxins, nutrients and enveloped viruses. In certain instances, the physiologic role of the acid environment has been well described (e.g. lysosomes, where a low pH is required for the activation of acid dependent hydrolases) whereas in other cases (e.g., endoplasmic reticulum) the role remains conjectural. What is certain is that acidic endomembrane proton pumps create such acid microenvironments in plants, fungi, yeast, mammalian cells, and, most pertinent, parasites, including Plasmodium falciparun (15,42).

A striking example of the role of endomembrane acidification in ligand receptor processing is seen in transferrin (43), as shown in Figure 14.

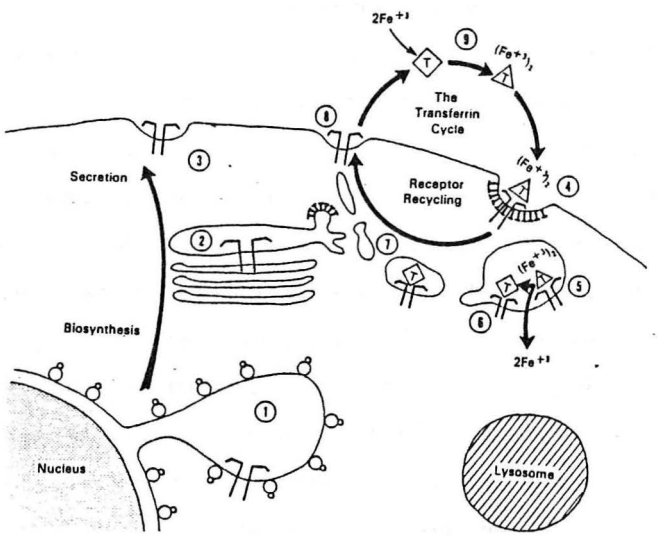

Figure 14: The Transferrin Processing Pathway. The transferrin receptor is synthesized in the endoplasmic reticulum (1) and is processed in the golgi (2) and transported to the cell surface (3). Circulating Fe-transferrin binds to the receptor and is endocytosed (4) and delivered to an endosome where endosomal acidity releases Fe (5 and 6). The transferrin receptor and transferrin are subsequently recycled (7-9).

Circulating transferrin carries two iron molecules. After binding to a specific plasma membrane receptor, the Fe-transferrin-transferrin receptor complex is internalized via the clathrin-coated pit-clathrin-coated vesicle system, and is delivered to the endosomal compartment. In this acidic environment, Fe, which is bound to transferrin through charge interaction, is leached from the transferrin molecule and ultimately is released into the cytosol where it utilized for a variety of metabolic processes. In experimental settings in which the acid environment of the endosome is disrupted, Fe is not released from transferrin and the cells become iron starved (43).

In addition to Fe-transferrin processing, dissipation of endomembrane acidification perturbs a variety of cellular functions: lysosomal proteases are mistargeted (44), glycosylation within the Golgi apparatus is perturbed (41), and, on a more positive note, cells become resistant to selected toxins (diptheria toxin, modeccin) (45) and to infection by encapsulated viruses (46). Experimentally, dissipation of organelle pH gradients is achieved by adding weak bases, such as NH_4Cl, to cell suspensions (47). The effect of such a weak base is depicted schematically in Figure 15. The base partitions into the vesicle, where it becomes protonated and is trapped, and in so doing, the base serves as a buffer with a resultant partial or complete dissipation of the pH gradient. Oftentimes, because of this trapping mechanism, the base is accumulated to such a high degree that an internal osmotic force is generated leading to vesicle swelling. Chloroquine is a diprotic weak base which has been used extensively as an experimental agent to disrupt endomembrane acidification (48).

In order to explain the role of chloroquine as an antimalarial agent, it is necessary to review a key stage in the life cycle - the intraerythrocyte

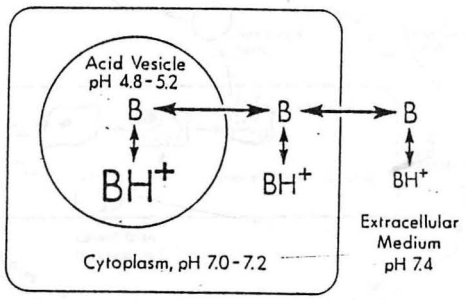

Figure 15: Alteration of Endomembrane Acidity by Weak Bases.

stage. During this period, the malarial organism passes from an early trophozoite to a mature schizont form, prior to lysis of the erythrocyte, as shown in Figure 16. During this developmental phase, the malarial organism is totally dependent upon the cytosolic contents of the red cell as a source of nutrients. Shown in Figure 17 is an electron micrograph of the malarial organism within the red blood cell. Notable is a large food vacuole or phagosome. The malarial parasite engulfs the cytosolic contents of the erythrocyte and proteins are transported to the phagosome where catabolism occurs. It has been demonstrated that the phagosome is acidic, and this acidity activates acid dependent hydrolases to release nutrient amino acids from ingested hemoglobin. Chloroquine, acting as a permanent base, enters the acidic compartment of the parasite, buffers the acid environment and prevents

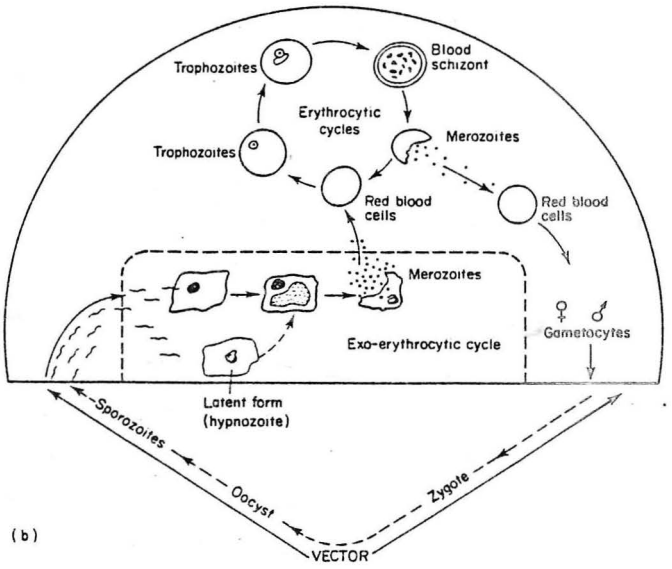

Figure 16: The Life Cycle of Malaria.

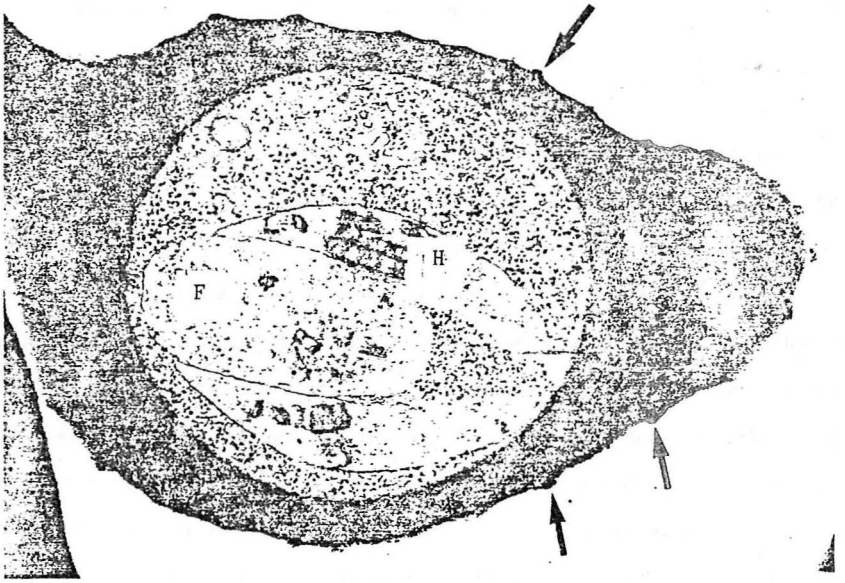

Figure 17: Electron Micrograph of an Intraerythrocyte Malarial Parasite. "F" refers to food vacuole, or phagosome, H refers to hemozoin pigment.

catabolism of nutrient proteins (42,49). Thus, phenomenologically, chloroquine's role as an antimalarial agent has been explained.

Two issues remain unresolved, however. First, why does chloroquine, which experimentally induces global dysfunction in cultured mammalian cells, exert its specific effect on the malarial phagosome in vivo? The answer lies in the doses of chloroquine used clinically and in the transport properties of the phagosome. Serum levels of chloroquine resulting from weekly dosages of 500 mg are insufficient to provide significant buffering to mammalian endomembrane compartments. The malarial phagosome, however, has an active concentrating mechanism which results in intraphagosome concentration of chloroquin to a level 1000-fold greater than would be predicted by its pKa (42). It is presumed that chloroquine accumulation occurs by a facilitated transporter situated in the phagosome membrane.

A second question in chloroquine's action exists regarding the nature of the nutrient which is becomes unavailable with buffering of the phagosome. It is becoming increasingly clear that the critical nutrient deficiency induced by chloroquine is iron. Returning to the electronmicrograph of Figure 17, electron dense particles (H) can be observed. These are referred to as hemozoin pigments and chemically they represent the heme moiety of hemoglobin. The malarial organism lacks heme oxygenase (50) and thus the iron of hemoglobin is not bioavailable to the organism (51). Recently it has been found that the malarial parasite is dependent upon Fe bound to transferrin as its source of elemental iron. Strikingly, it has been found that the malarial parasite synthesizes its own transferrin receptor, which is transported from the parasite and inserted into the erythrocyte plasma membrane. In this setting, the illicit receptor scavenges circulating Fe-transferrin and the organism evidently extracts this complex, directly or indirectly from the

erythrocyte plasma membrane (52). The critical need for an acid environment in the processing of Fe transferrin was discussed previously, and thus the possibility arises that the antimalarial activity of chloroquine relates rather specifically to its disruption of the pH-dependent processing of the Fe-transferrin complex.

In this regard, clinical studies in Africa have suggested that chronic, low grade iron deficiency may be protective against malarial infection. Moreover, iron replacement therapy in tribes living in areas endemic for malaria is associated with an increase in the frequency of malaria (53). Further support for this notion comes from experimental observations of Chinese hamster ovarian cells which bear a mutation in their endomembrane proton pumps such that endosomal acidification is impaired. This defect has been found to be conditionally lethal, and can be overcome solely by increasing iron within the incubation media (54,55).

In summary, chloroquine's chemoprophylactic activity against Plasmidia has been demonstrated to be due to its effect as buffer, which, when concentrated within the phagosome, results in an elevation of pH and an inability to hydrolyze nutrient protein and supply iron to the organism.

OMEPRAZOLE: A GROUP SPECIFIC INHIBITOR WITH DIVERSE EFFECTS

In the remainder of this monograph, the effects of a relatively new inhibitor of plasma membrane ATPases will be reviewed. Although omeprazole is a group-specific (sulfhydryl) inhibitor, it has been shown to inhibit two distinct types of proton pumps: the gastric mucosa, E_1E_2 type H^+K^+ ATPase, and the endomembrane type proton translocating ATPase. Very different therapeutic implications arise from the inhibitions of these two systems and omeprazole has proven efficacy in the treatment of peptic ulcer disease and

preliminary data indicate it is of potential benefit in the treatment of osteoporosis.

OMEPRAZOLE AS AN INHIBITOR OF GASTRIC ACID SECRETION

The parietal cells of the gastric mucosa are responsible for the HCl secretion, and, indeed, the steepest ion gradients observed in mammalian systems occur in the stomach where, under maximal acidifying conditions, a luminal pH of 0.5 can be generated, leading to a 10 million-fold proton gradient from lumen to cell interior.

From the standpoint of cellular physiology, the mechanism of gastric secretion is well described (56), as illustrated in Figure 18. Under basal conditions, the acid secreting cell of the stomach, the parietal cell, has a structure consisting of a relatively simple apical border and a collection of intracellular vesicles (tubulo-vesicular bodies) which reside beneath the plasma membrane (57). Studies conducted with pH sensitive dyes indicate that these tubulo-vesicular bodies are acidic. With activation of gastric mucosal glands by histamine, a complex cellular cascade is set into operation. As a result of occupancy of the histamine H2 receptor, intracellular Ca^{2+} and cyclic AMP levels rise and this results in the fusion of the acidic tubulovesicular bodies with the apical surface to form a complex invagination termed the canniliculus. Concordant with the development of the canniliculus is an increase in the gastric proton secretory rate (58).

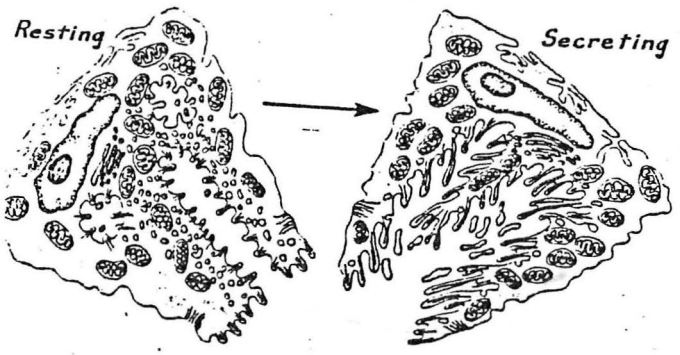

Figure 18: Morphologic Transformation in Gastric Parietal Cell during Histamine Induced Activation.

The final effector of gastric acid secretion is an E_1E_2 type ion translocating ATPase, the H^+K^+ ATPase (59). This enzyme is composed of a single polypeptide chain of molecular mass of 96 kDa. It catalyzes the secretion of protons and the reabsorption of potassium at a stoichiometric ratio of 1 mole H^+/1 mole K^+/1 mole ATP (60), as shown in Figure 19.

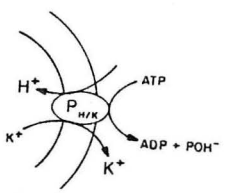

Figure 19: Model of the Gastric Mucosa H^+K^+ ATPase.

Immunocytochemical and biochemical studies have localized the H^+K^+ ATPase to tubulovesicular bodies in the resting state, and to the apical surface (cannilicular surface) in the activated state (61). Characterization of the H^+K^+ ATPase has included the cloning and sequencing of its cDNA, and

structurally, this enzyme closely resembles the α chain of the Na^+K^+ ATPase, with intense conservation of the ATP binding and phosphorylation sites, and a general conservation of the 7-8 transmembranous sections. Differences among the two pumps are found in the amino acid composition of these transmembranous sectors, presumably serving to impart ion specificity to the species which are transported (62).

Although a number of anti-ulcer agents are of clinical benefit (including carbonic anhydrase inhibitors) the most popular therapeutic agents at present operate as rather specific histamine H2 receptor antagonists and include cimetidine and ranitidine. These agents operate by blocking activation of parietal cells at the level of the receptor. Recently, however, a more distal inhibitor of gastric acid secretion, omeprazole, has been developed, which reacts directly with the H^+K^+ ATPase.

Omeprazole belongs to a general class of substituted benzimidazole sulfoxides. Shown in Figure 20 is the full structure of the most clinically useful compound, omeprazole. All members of this group serve as sulfhydryl reactive agents. As many enzymes have sulfhydryl and often, critical sulfhydryl groups, it would be expected that omeprazole would be a very non-specific reagent and not

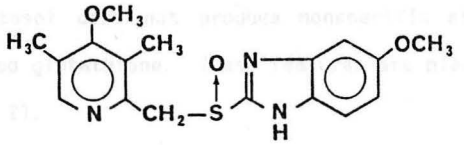

Figure 20: Structure of Omeprazole.

useful as a therapeutic agent. Specificity in its action, however, is lent by the fact that the parent compound is inactive, and requires exposure to an acidic environment for activation. Under such conditions, omeprazole (pKa = 4) is trapped in acidic compartments and undergoes cyclization to generate an active SH reactive group (63).

It had been previously observed that purified H^+K^+ ATPase is inhibited by SH reactive agents, such as N-ethylmaleimide (NEM) (59). The first description of the inhibitory effect of the -prazole type inhibitors occurred by chance, wich the observation that timoprazole inhibited gastric acid secretion. Subsequently, it was demonstrated that this compound inhibited the H^+K^+ ATPase itself, but only in acidifying vesicle systems (64). This led to the realization that the compound required acid activation. Later, the structure of this parent compound was modified to yield omeprazole, an agent with certain advantages over timoprazole, notably a lack of thyro- and thymotoxicity.

Thus, omeprazole's utility can be traced to two essential structural features. First, because it has a pKa of about 4, it is concentrated in extremely acidic compartments and second, the compound requires acid activation which imparts selectively to what would otherwise be a nonspecific sulfhydryl reactive reagent. Moreover, the effects of omeprazole are prevented by reducing agents and thus penetration of the compound into the parietal cell cytosol does not produce nonspecific effects because of the presence of reduced glutathione. These features are placed in a physiological context in Figure 21.

At present, the exact sites of omeprazole's action in the gastric H^+K^+ ATPase have not been defined, although candidate SH groups have been identified in the predicted primary structure of the enzyme (62), and the

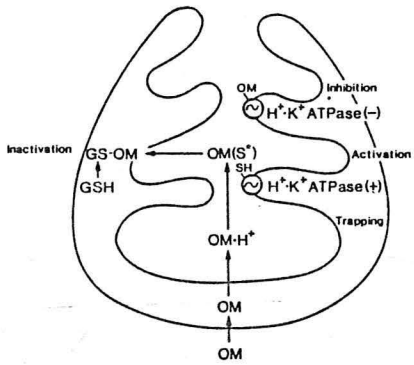

Figure 21: A Model of the Action of the Acid Secretion Inhibitor Omeprazole. This shows acid gradient-dependent accumulation of the weak base, acid activation, and reaction with an SH group of the H^+-K^+ ATPase. Any cellular access of acidic compound reacts with glutathione, rendering it harmless.

stoichiometry of omeprazole binding to the H^+K^+ ATPase is between 1 and 2 (65).

Clinically, omeprazole is effective in the treatment of peptic ulcer disease and currently is utilized as an agent in the treatment of cimetidine-refractory Zollinger-Ellison syndrome (66,67). After administration of either oral or parenteral omeprazole, inhibition of gastric acidification occurs for over 30 hours, as the H^+K^+ ATPase is, under these conditions, irreversibly inhibited and release of the inhibitory effects occurs only with the de novo synthesis and insertion of H^+K^+ translocating ATPases (68).

OMEPRAZOLE AS AN INHIBITOR OF OSTEOCLAST-MEDIATED BONE REABSORPTION

As noted in the discussion of chloroquine, all cells contain a variety of acidic intracellular compartments. In addition, the luminal contents of the distal nephron can achieve a pH of 4.5 owing to action of an electrogenic proton translocating ATPase, which is an endomembrane type ion translocating pump (15). To date, there have been no conclusive clinical reports of an illicit action of omeprozole in producing either inhibition of endomembrane

acidification or a distal renal tubular acidosis. However, the utility of omeprazole, or a future derivative, in the prevention of osteoporosis is under current investigation. The rationale for this approach is detailed below.

Bone remodeling and reabsorption involves a complicated interplay of resident surface osteoblasts and osteoclasts. For the purposes of this monograph, focus will be placed upon the most distal aspect of the overall process, the cellular mechanism of osteoclast-mediated bone reabsorption, and specifically, the mediation of bone dissolution by an endomembrane type proton translocating ATPase.

Osteoclast physiology can be subdivided into three stages (69). The first involves the formation of osteoclast precursors in bone marrow, followed by their spread and formation of preosteoclasts and osteoclasts which are resident in bone (70,71). The second phase includes the activation of osteoclasts, a step which is critically dependent upon osteoblast-osteoclast interactions (69,71-73). Shown in Figure 22 is a schematized view of this interaction. It is important to note that osteoclast activation is essentially controlled by the osteoblast. Several theories, including shielding of bone surface from osteoclast activity by osteoblasts have been put forward. At present, however, evidence is clear that the activation of the the osteoclast requires a signal transmission from the osteoblast. In this regard, it has been shown that PTH action in vitro requires the co-culturing of osteoblasts and osteoclasts; no effect of PTH on isolated osteoclasts is observed under these conditions (74).

The activation of osteoclasts is accompanied by striking morphologic changes in the osteoclast. Like the gastric parietal cell, the "apical" or bone surface of the osteoclast undergoes extensive expansion in formation of a ruffled border and, in addition, the osteoclast becomes sealed to the bone to

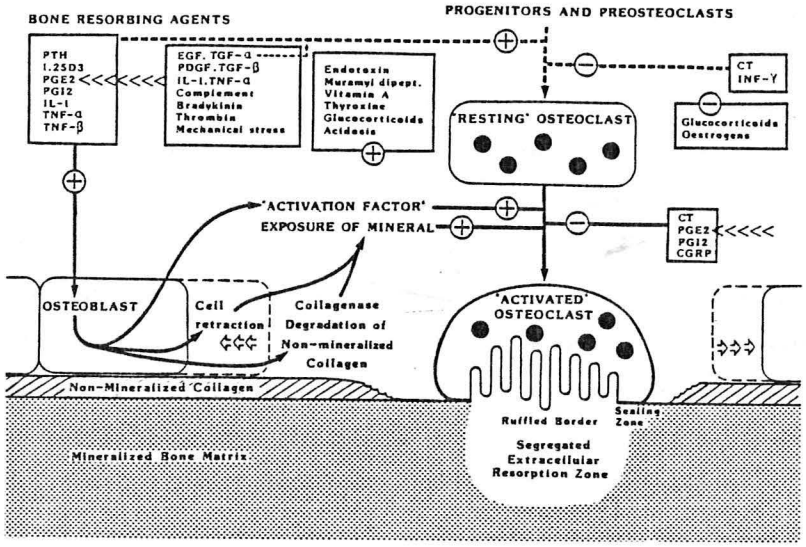

Figure 22: Hypothetical View of Osteoclast Activation.

produce a microenvironment for cellular action (75,76).

Reabsorption of bone by the osteoclast can be subdivided into two discrete processes: breakdown and reabsorption of mineral (essentially hydroxyapatite) and catabolism and reabsorption of matrix (essentially collagen). Both processes require an acid microenvironment. This is achieved by acidification of the sequestered space between the osteoclast and bone, which has been determined to have a pH of 4.0-5.0 (77). Cellular and transport events in the acidification process are depicted below in Figure 23. Acidification of the space occurs by means of a proton translocating ATPase which is situated on the ruffled border (78). The acidity itself is responsible for the dissolution of hydroxyapatite (which chemically has been

shown to break down in an acid environment) and the released Ca^{2+}, Mg^{2+} and $PO_4^{=}$ transverse the cells in an ionic, rather than fragmented form, by an unknown mechanism. Catabolism of collagen occurs by means of release of lysosomal contents into the acidic microenvironment of the bone-osteoclast interface. Available evidence indicates that the critical collagen degratory enzymes are acid-activated cysteine proteinases. The resultant low molecular weight peptides are subsequently reabsorbed by the cell (80).

Of most importance to this discussion is the nature of the proton ATPase responsible for acidification of the sequestered microenvironment. Immunohistochemical evidence (78) indicates that this is an endomembrane type proton pump of the type responsible for endosomal and urinary acification. As noted in Table I, these proton pumps, unlike the gastric mucosa H^+K^+ ATPase,

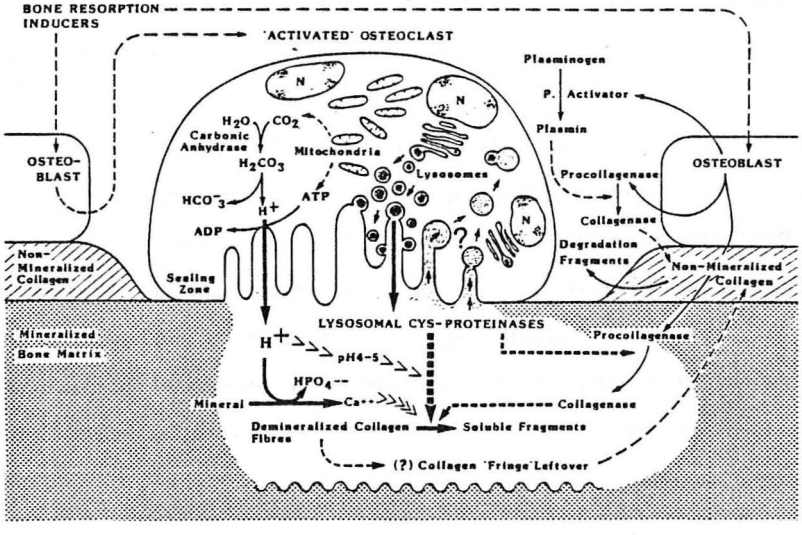

Figure 23: Model of Bone Reabsorption of Osteoclasts.

are large, multisubunit systems. Characterization of these pumps has revealed that they are composed of two general sectors, one responsible for ATP hydrolysis and the other for transmembranous proton movement (15). The proton pore of one of these systems has been resolved (81).

Recently, it has been demonstrated in vitro that omeprazole can block bone reabsorption, and this opens the opportunity for the investigation of this, or derived compounds, in the treatment of osteoporosis (82). The endomembrane proton pump is functionally and structurally very different from the gastric H^+K^+ ATPase, and the finding that omeprazole can have an inhibitory effect on an endomembrane proton pump is likely explained by the nonspecificity of its sulfhydryl-reactive group when activated. Thus, as with gastric mucosa, omeprazole is activated by the acidic environment of the osteoclast-bone interface and the liberated SH reactive group interacts with an accessible portion of the endomembrane pump present on the ruffled border. The inhibition of this type of proton pump by omeprazole has been shown to be time and pH dependent, and available evidence indicates that omeprazole interacts with the proton pore of the pump (83).

At present, omeprazole is undergoing in vivo studies in animals to assess its utility as an anti-osteoporotic agent. Potential pitfalls include inhibition of the gastric H^+K^+ ATPase as well as other endomembrane pumps beyond that of the osteoclast. It is likely that the present compound will require modification to allow for site specificity. In addition, advancing knowledge of the structure of endomembrane proton pumps holds the potential for delineation of tissue-specific isozymes, to which the drug can be tailored.

SUMMARY

Several examples of a general group of therapeutic agents directed against ion translocating ATPase have been reviewed. The selectivity and mode of action of two well-known agents, digoxin and chloroquine, have been largely explained. In both instances, the therapeutic selectivity of action was appreciated long before the mechanism of action was explained. In addition, a more recently developed agent, omeprazole, has been discussed as an example of a compound which can have designed selectivity and whose nonselectivity opens new therapeutic approaches. All of these compounds hold in common the effect of blocking the most distal event in physiologic cellular processes and underscore the utility of transport inhibitors as a potent class of therapeutic agents.

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