Physiological Chemistry and Physics and Medical NMR, 19 (1987)

AN ELECTRONIC MECHANISM IN THE ACTIONS OF DRUGS AND OTHER CARDINAL ADSORBENTS. I. EFFECT OF OUABAIN ON THE RELATIVE AFFINITIES OF THE CELL SURFACE β- AND γ- CARBOXYL GROUPS FOR K', Na^+, GLYCINE AND OTHER IONS

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After more than three decades of experimental testing these basic postulates have been confirmed:

I. extensive counterion association following charge fixation (Kern, 1948; Ling, 1962, p. 17; 1984, p. 151; Ling and Zhang, 1983);

2. fixed anionic β- and γ-carboxyl groups of proteins in general have the inherent capability of selective adsorption of alkali metal ions when competing fixed cations are removed (Ling and Zhang, 1984);

3. the bulk of cell K' is in an adsorbed state (Ling, 1984, p. 227; Edelmann, 1984) on β-and γ-carboxyl groups (Ling, 1984; a more complete proof can be found in Ling, 1988, Section 4.4.).

4. the distribution of K' (and Na^+) follows the predicted quantitative relationships of the association-induction (AI) hypothesis (Ling, 1984, p. 319).

Ling also suggested in 1952 that qualitatively speaking, the cell membrane, like the cytoplasm, also contains β- and γ-carboxyl groups-carrying proteins. As such, the cell surface should behave toward K' and Na^+ in a way similar to the bulk phase cytoplasm. That is, under normal resting conditions, the β- and γ-carboxyl groups of the cell surface membrane should also be associated predominantly with K^+. This selectivity of K' over Na' in adsorption onto the β- and γ-carboxyl groups on the cell surface was then suggested as the key mechanism for the preferential permeability of the cell surface membrane to K' over Na^+. Ion permeation is seen as involving the sequential steps: (i) successful occupancy of the fixed β- and γ-carboxyl groups on the cell surface by K', followed by (ii) libration of the adsorbed K' around the fixed anion and (iii) dissociation of the K' from the fixed anion and entry into the cell. While this ***adsorption-desorption route*** accounts for the major share of K' entry (ca. 90%, see Ling, 1984; for more detailed data see Ling, 1988, Figure 94), other K' enters the cell through domains of the cell membrane comprising strongly polarized water, without undergoing the association-dissociation process ***(saltatory route).*** The saltatory route is a more important route for ions less preferred by the fixed anion, like Na^+, accounting for some 30% to 50% of its traffic (see Ling, 1984; see also Ling, 1988, Figure 94).

As a general theory of the living cell, the association-induction (AI) hypothesis does not merely deal with the mechanism of diverse physiological manifestations such as selective solute distribution, permeability, electric potentials, but also with the mechanism underlying the reversible changes of the cells between the resting and active states, between the living and the dead state, etc., in response to the presence or absence of highly potent molecules, referred to as "cardinal adsorbents", of which drugs are examples.

To explain specificity of drug action, Paul Ehrlich introduced the famed lock-and-key analogy. Lock-like receptor sites respond

only to drugs which have the right molecular structures and steric configuration to fit the receptor sites. Yet a key that fits a lock is as useless as one that does not fit the lock, unless the "fitting" key is "turned". Thus, it is the **"turning"** that opens the door. A survey of textbooks reveals a virtual blank on this "key turning" issue. It was to fill this void that an electronic polarization or inductive mechanism for drug action, as well as the action of other biologically potent cardinal adsorbents was offered in the **AI** hypothesis.

The inductive effect, formally introduced by G. N. Lewis in 1923 has long been a part of the fundamental theory of organic chemistry. A vast number of equilibrium and kinetic properties of (small) organic molecules has been explained on the basis of the inductive theory (Ingold, 1953; Hammett, 1970; Chiang and Tai, 1985). It seemed logical and natural that what operates in small organic molecules, must continue to operate in larger ones like proteins which are small organic molecules joined together.

According to the **AI** hypothesis, the binding of a drug or other ligand onto a suitable "receptor site" may alter electronic conformation of the protein by modifying the inductive effects exercised by the protein's own amino acid side chains. Such an inductive effect produced by ligands is either directly transmitted to a target group close by, or indirectly to remote sites by what is called an indirect **I-effect.** This type of falling-domino-like interaction underlies a type of physical behavior collectively known as "cooperative" phenomena (Ling, 1964, 1980, 1984).

In the **AI** hypothesis, a parameter called the c-value was introduced. The c-value measures primarily the electron density at the singly charged oxygen atom of the carboxyl (or other oxyacid) group and is in units of Angstrom. A low c-value corresponds to a low pK_a value; a high c-value corresponds to a high pK_a value. In conventional usage, the difference in the acid dissociation constant expresses how c-value-changes alter the affinity of one countercation, i.e., H. However, Ling also computed the relative adsorption energies of the five alkali-metal ions (Li', Na', K^+, Rb', and Cs') in addition to NH_4^+ and H' at varying c-values, using known laws governing electronic interaction and physical constants available in the literature. The result of computation is illustrated in Figure 1 which shows that the rank order of selectivity of K^+ and Na^+ as well as the other ions changes with the c-value change.

Returning to the lock and key analogy, one can say that in the **AI** hypothesis the inductive effect acts as the equivalent of the "turning" of a fitting key.

Drugs, and other cardinal adsorbents, can be divided into four classes:

1. drugs that do not bind onto receptor sites;

2. drugs that bind onto receptor sites but produce no electron-perturbation (electron-indifferent-cardinal adsorbent or EIC);

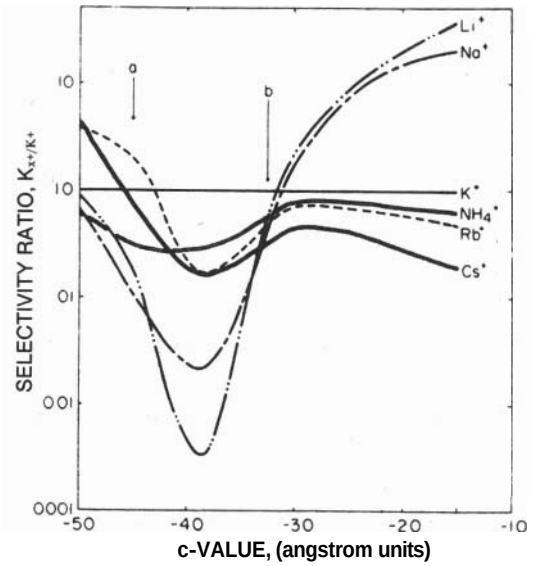

FIGURE 1. The relation between the selectivity ratios of various cations and the c-value. The K^+ ion is taken as unity and selectivity ratios are calculated from the association energies given in Figure 4.11 of Ling (1962) (polarizability of anionic group: 2.0×10^{-24} cm^3). (Ling and Bohr, 1971)

3. drugs that bind onto receptor sites producing an electron-withdrawing effect (electron withdrawing cardinal adsorbent, EWC); and

4. drugs that bind onto receptor sites producing an electrondonating effect (electron-donating cardinal adsorbent, EDC). Usually a specific physiological response is the consequence of a change of c-value from a resting value to an active value. This may be achieved by either an EDC or an EWC.

These theoretical concepts have led to the prediction that interaction with an EDC, will produce an increase in the electron density of the target β- and y-carboxyl groups corresponding to a rise of the c-value and that interaction with an EWC will create an overall decrease of the electron density of the target β- and y-carboxyl groups corresponding to a fall of the c-values. The c-value rise and fall can be roughly recognized by an increase or decrease respectively of the ion selectivity ratio, $K_{Na+/K+}$ (as represented by the ordinate of Figure 1). More exact determination of the c-value rise or fall can be obtained by determining the selectivity ratio of not just a single pair of ions (e.g., Na^+ vs. K) but of several pairs of ions (e.g., Li^+, Na', Rb^+, Cs' vs. K'). Indeed our strategy was to study the binding energies of the five alkali-metal ions in addition to glycine onto the surface β- and γ-carboxyl groups, in order to answer the key question if the drug ouabain controls ion permeability by altering the c-value of the β- and y-carboxyl groups and if so, is ouabain an EDC or EWC?

MATERIALS AND METHODS

All experiments were performed on the isolated sartorius muscles of leopard frogs (Rana pipiens pipiens) obtained from Vermont. The frogs were routinely kept on arrival in a large stainless-steel tank in tap water containing tetracycline (0.8% w/v) for one week then changed to a running water sink and force fed canned dog food twice a week.

All chemicals used were of C.P. grade. Ouabain was obtained from Sigma Chemical Co., St. Louis, MO, (Lot # L5F-0551).

The sartorius muscles used were isolated on the day prior to the experiment, and stored overnight at 4°C in a Ringer's phosphate solution (NaCl, 92.7 mM; KCl, 2.5 mM; $CaCl_2$, 1.0 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 6.6 mM; NaH_2PO_4, 2.0 mM; Na_2HPO_4, 1.2 mM; D-glucose, 24 mM).

As a rule two sartorius muscles each from a different frog were preincubated for 4½ hours in 25 cc normal Ringer's phosphate solution (with or without 10^{-6} M ouabain) in

TABLE I. Makeup of the incubation solutions from various stock solutions. NRP, normal Ringer's phosphate solution, for composition see text. Solution I contains NaCl(46.4 mM); $CaCl_2$ (5 mM); $MgSO_4$ (8.6 mM); Na Hepes, Na (60 mM); pH = 6.8. Low pH is maintained to prevent crystallization of Cs^+ salts. XCl refers to LiCl, NaCl, KCl, RbCl, CsCl or glycine HCl.

$[Cs^+]_{ex}$ (mM)		NRP (ml)	Ouabain (10^{-4} M) in NRP (ml)	Solution I (ml)	XCI (0.118 M) (ml)	Cs*Cl (0.118 M) (ml)	Sorbitol (0.118 M) (ml)
2.0	Control	0.075	—	1.16	5	0.125	1.125
	Ouabain	—	0.075	1.16	5	0.125	1.125
10.0	Control	0.075	—	1.16	5	0.625	0.625
	Ouabain	—	0.075	1.16	5	0.625	0.625

250 ml Erlenmeyer flasks which were placed in a constant temperature room (25° ± 1°C) and shaken gently on a rotary shaker at 75 rpm.

After preincubation and gentle blotting, the muscles were incubated at 25°C in 7.5 ml of incubation solution containing 80 mM of a non-labeled alkali-metal ion or glycine and 2 mM or 10 mM ^{134}Cs-labeled CsCl, vigorously bubbled with air. The compositions of the incubation solutions are given in Table I. After 11 minutes of incubation, the muscles were taken out and fluids trapped in the extracellular space removed by the centrifugation method of Ling and Walton (1975). Briefly, the muscles were placed with their shiny (dorsal) side up on 2 layers of filter paper that had been moistened with the tagged incubation solution. These decks of filter paper with their muscles were in turn placed on top of four more layers of filter paper which had been prewetted by autoclaving in steam for 5 minutes. Two layers of similarly autoclaved filter paper were placed on top of the muscle, and the whole assembly wrapped and hermetically sealed in parafilm in a humidity chamber. The packets were placed at the bottom of 250 ml aluminum centrifuge cups with flat bottoms, making sure that the shiny side of the muscle remained in the up position, and centrifuged at 1000 g. for 3-4 minutes. The muscles were then removed from the packets and frozen in liquid nitrogen, weighed while frozen and then projected into counting tubes containing 1 ml. of 0.1 N HCl before their radio-activity was assayed on an automatic gamma counter.

METHODS OF DATA ANALYSES

The primary purpose of this research is to determine if exposure to ouabain alters the

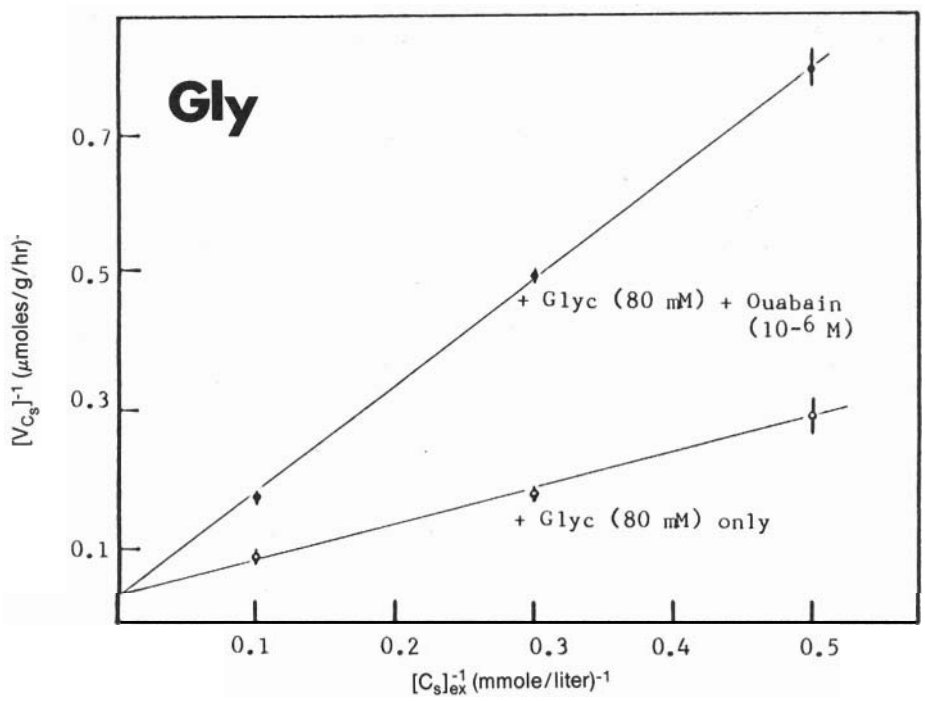

FIGURE 2. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM glycine in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

relative binding affinities of the five alkali-metal ions on the surface β- and γ-carboxyl groups. To achieve this goal we determined the rate of entry of labeled Cs^+ into frog sartorius muscles in the presence of a fixed concentration of a competing ion in the absence and presence of ouabain.

As mentioned above, the main route of Cs' entry into frog muscle is via the adsorption-desorption route. In the case where K' is also present in the external medium, the rate of entry of labeled Cs' into the cells (V_{Cs}) is given by the following equation (Ling, 1953, 1955, 1962, p. 297; Ling and Ochsenfeld, 1965):

$$V_{Cs} = \frac{V_{max}\,[Cs^+]_{ex}\,K_K}{1 + [Cs^+]_{ex}\,K_{Cs} + [K^+]_{ex}\,K_K}, \qquad (1)$$

which can be written in the reciprocal form:

$$\frac{1}{V_{Cs}} = \frac{1}{V_{max}}\left(1 + K_K \frac{[K^+]_{ex}}{K_{Cs}}\right)\frac{1}{[Cs^+]_{ex}} + \frac{1}{V_{max}}. \qquad (2)$$

$[Cs^+]_{ex}$ and $[K^+]_{ex}$ are the external Cs^+ and K' concentration respectively and K_C, and K_K their respective adsorption energies on the cell surface β- and y-carboxyl groups (see Ling and Ochsenfeld, 1965). By plotting $1/V_{Cs}$ against $1/[Cs^+]_{ex}$, one expects to find a family of straight lines converging on the same locus on the ordinate with slopes varying with $[K^+]_{ex}$ and intercepts equal to the reciprocal of the maximum rate of entry via the adsorptiondesorption route (V_{max}). From V_{max} and the slopes of the straight lines in the

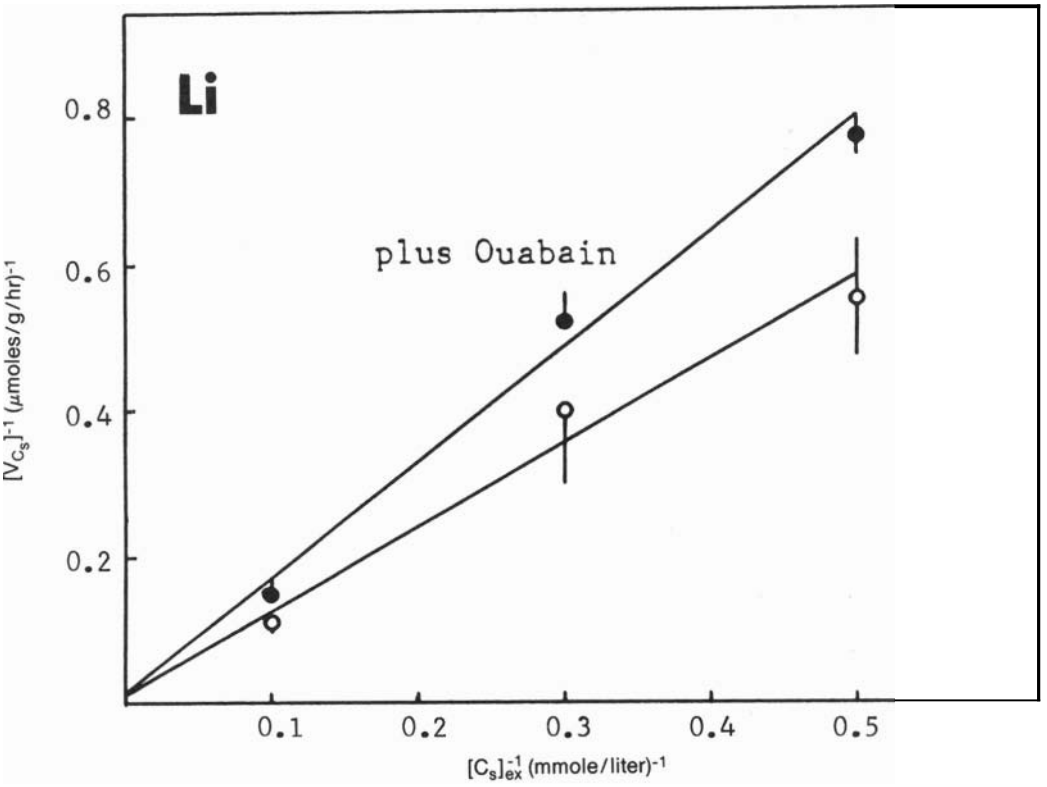

FIGURE 3. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM Li in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

absence and presence of $[K^+]_{ex}$, one obtains both K_{Cs} and K_K.

RESULTS AND DISCUSSION

We studied the entry of ^{134}Cs – labeled Cs' into isolated frog sartorius muscle cells at two different Cs^+ concentrations (2 and 10 mM) in the presence of 80 mM glycine. In Figure 2 the reciprocals of the rate of labeled Cs^+ entry in the absence and presence of 3.27 $\times 10^{-7}$ M ouabain were plotted against the reciprocals of the labeled Cs^+ concentration according to Equation 2. This difference in slopes of the data of the ouabain-treated muscles and their controls indicates that ouabain treatment had significantly enhanced the effectiveness of glycine in competing for

TABLE II. Effect of ouabain on the inhibiting effect of 80 mM of glycine, Li^+, Na^+, K^+, Rb^+, and Cs^+ on the rate of entry of labelled Cs^+ (2.00 mM and 10.00 mM) into frog sartorius muscles.

$[Cs^+]_{ex}$ (mM)	Competing ion	V_{Cs} (μmoles/gram/hour)					
		Cs	Rb	K	Na	Li	Gly
2.00	Control	1.55 ± 0.12	1.73 ± 0.28	1.31 ± 0.09	1.82 ± 0.102	1.94 ± 0.28	3.39 ± 0.27
	Ouabain	1.74 ± 0.14	1.50 ± 0.07	1.45 ± 0.13	1.50 ± 0.06	1.30 ± 0.05	1.26 ± 0.05
10.00	Control	7.83–0.45	7.04–0.17	7.10 ± 0.17	9.41 ± 0.67	9.26 ± 0.85	10.91 ± 1.28
	Ouabain	8.24 ± 0.19	7.54 ± 0.59	6.88 ± 0.43	7.49 ± 0.35	7.04 ± 0.82	5.47 ± 0.13

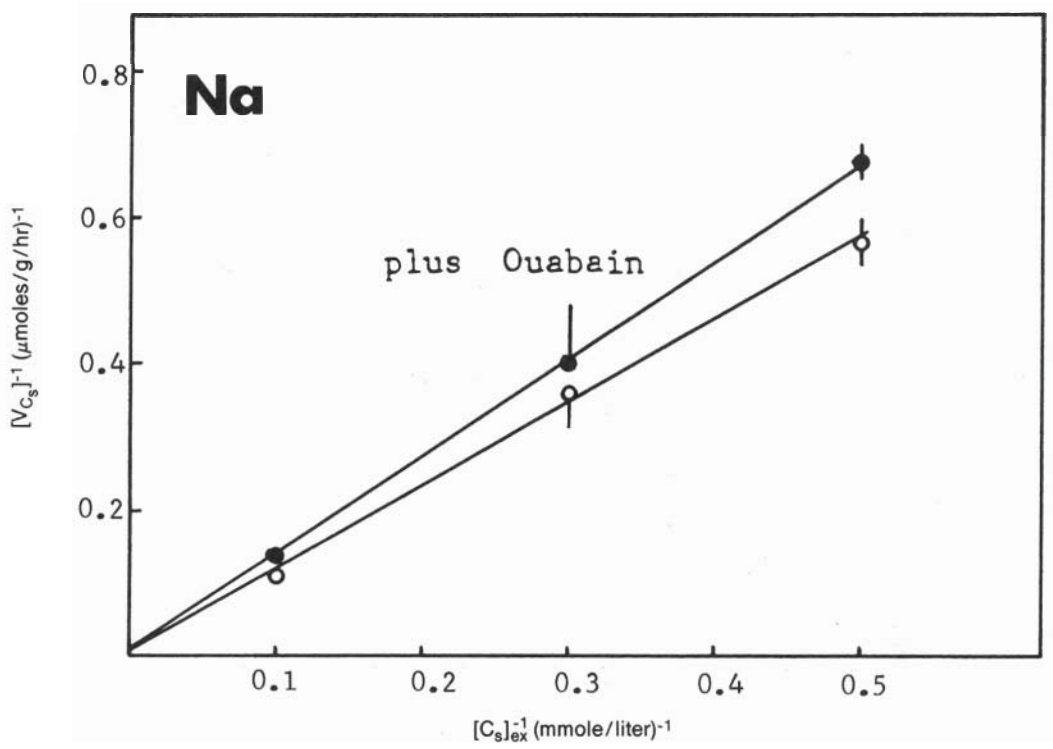

FIGURE 4. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM Na in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

and thus reducing the rate of entry of labeled Cs' into the frog muscle cells. The convergence of the two curves on the same locus on the ordinate is in harmony with the view that ouabain treatment did not create new anionic sites that mediate Cs' entry or eliminate old ones.

The same format used in generating the data of Figure 2, was used in comparing the relative affinity of Li', Na^+, Rb', Cs', and K' with that of Cs^+ in response to ouabain. The results are given in Figures 3 to 7 and summarized in Figure 8. Table II presents the numerical values of the data shown in Figure 8, where the rate of entry of 2 mM labeled Cs' was studied, in addition to data from studies of Cs' entry at 10 mM external concentration. The responses observed were essentially similar at 2 mM labeled Cs' concentration as 10 at mM. Figure 8 and Table II also show that the rate of Cs' entry into normal control muscle was highest in the presence of 80 mM glycine, next highest in the presence of 80 mM Li', etc., suggesting that the β- and y-carboxyl group mediating Cs' entry shows the rank order of adsorption preference: K' > Cs', Rb' > Na', Li' > glycine. After treatment with 10^{-6} M ouabain, the relative adsorption energies among these ions are by and large reversed. The new rank order is now: glycine, Li^+, Na^+ > K', Rb' > Cs^+.

Comparing the experimental data with the theoretical curves shown in Figure 1, one concludes that the ouabain alters the c-value of the muscle cell surface β- and γ-carboxyl groups and in such a direction that indicates that ouabain acts as an EDC.

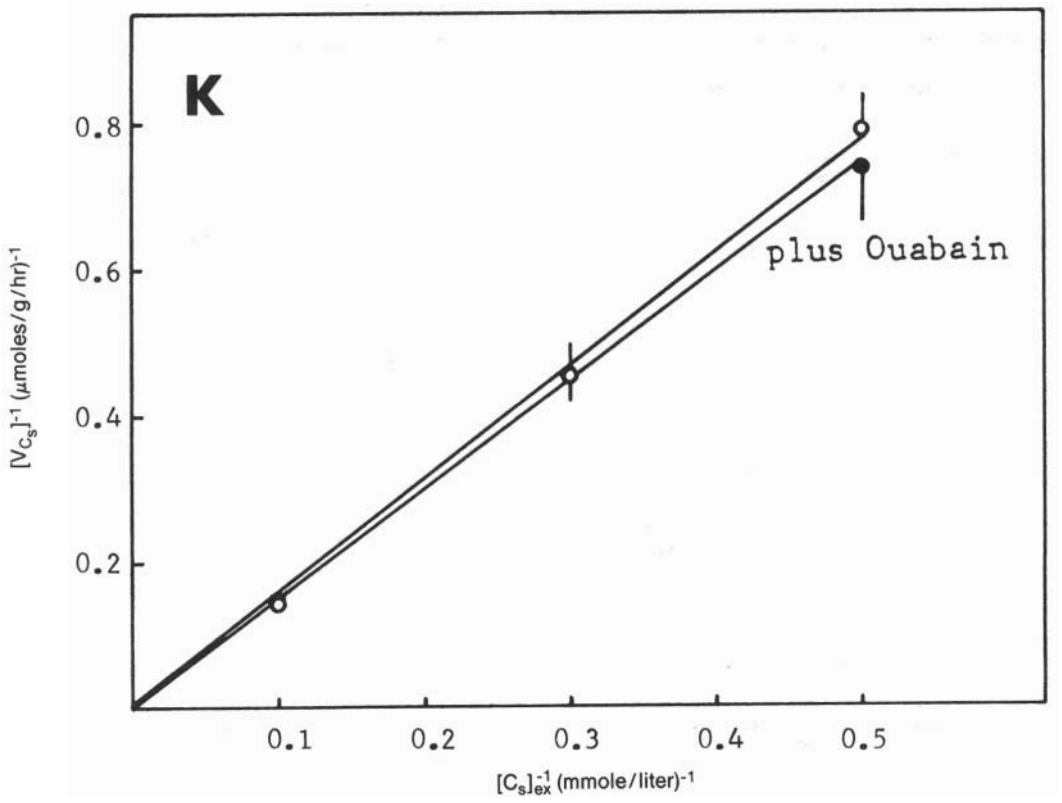

FIGURE 5. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM K in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

DISCUSSION

Digitalis represents a very important group of drugs, often referred to as cardiac glycosides, that have powerful action on the myocardium and is extensively used clinically for the treatment of congestive heart failure. In 1953 Schatzmann reported that in human red blood cells stored in the cold the regain of K and loss of Na^+ were inhibited by cardiac glycosides. Since then cardiac glycosides in general and ouabain in particular have gained wide use as a specific inhibitor of the postulated Na pump.

For the documentation of the disproof of the membrane-pump theory, the reader is advised to consult Ling, 1984, however, a more detailed disproof can be found in Ling, 1988 (see in particular Chapter 12 for full summary). More specifically it may be mentioned that the effect of ouabain on the equilibrium distribution of frog muscle is independent of the existence of a functional cell membrane (and the postulated Na pump in the cell membrane) (Ling, 1973, 1978) and that the control of K^+ and Na^+ distribution in a variety of living tissues studied follows quantitatively the theory of controlled cooperative adsorption of K and Na^+ (see Ling, 1984, p. 358; Gulati and Jones, 1971; Negendank and Collier, 1976).

Furthermore, the ouabain action on K^+/Na^+ distribution strongly resembles the effect of 2,3-diphosphoglycerol (DPG) on the binding of oxygen on hemoglobin; in both, the drug alters the relative affinity of ligands that bind onto remote sites. At a concentration of 3.27×10^{-7} M the ouabain effect on the equilibrium K^+/Na^+ distribution in frog muscle is largely a consequence of a rise of the binding energy of Na^+ in comparison with K^+. The intrinsic equilibrium exchange con-

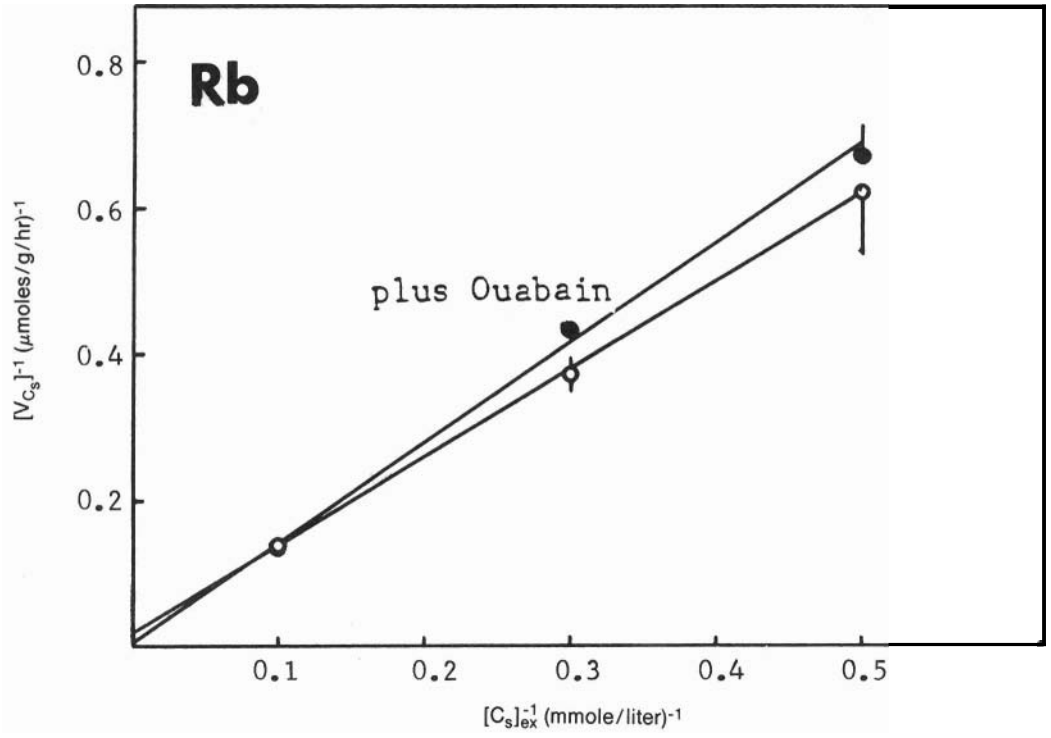

FIGURE 6. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM Rb in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

stant K^{∞}_{Na-K} dropped from 100 to about 20 (Ling and Bohr, 1971). This change of K^{∞}_{Na-K} could occur as a result of both c-value rise or c-value fall of the β- and y-carboxyl groups because in the theoretical curves (Figure 1), the ratio of the adsorption constant for a pair of ions is not always uniquely defined.

However, the c-value of a whole set of ratios among a multitude of ions are uniquely defined as Ling and Bohr's study of the equilibrium distribution of the five alkali-metal ions in frog muscles has demonstrated (Ling and Bohr, 1971). The changes observed can be matched reasonably well with the assumption that anion groups adsorbing these ions have shifted their c-value from a value of –4.50 Å (indicated by "a" of Figure 1) to –3.25 Å (indicated by "b" of Figure 1). Since this work was published in 1971, major advances have been made which have established unequivocally that in frog muscle cells, K^+ and other alkali-metal ions are indeed adsorbed on the β- and y-carboxyl groups localized at the edges of the A bands and Z-lines (Ling, 1984, p. 232), further increasing the significance of these older findings of Ling and Bohr. They have clearly demonstrated that the drug ouabain at a low concentration (3.27×10^{-7} M) is capable of increasing the c-value of a large number of β- and y-carboxyl groups in a cooperative manner as an electrondonating cardinal adsorbent or EDC.

According to the AI hypothesis, the basic molecular mechanisms underlying a variety of physiological behavior of the living cells are highly similar.

Thus if the same β- and y-carboxyl groups at the cell surface that mediate K^+ entry into cells also gives rise to the resting potential, as demonstrated by the important experiment

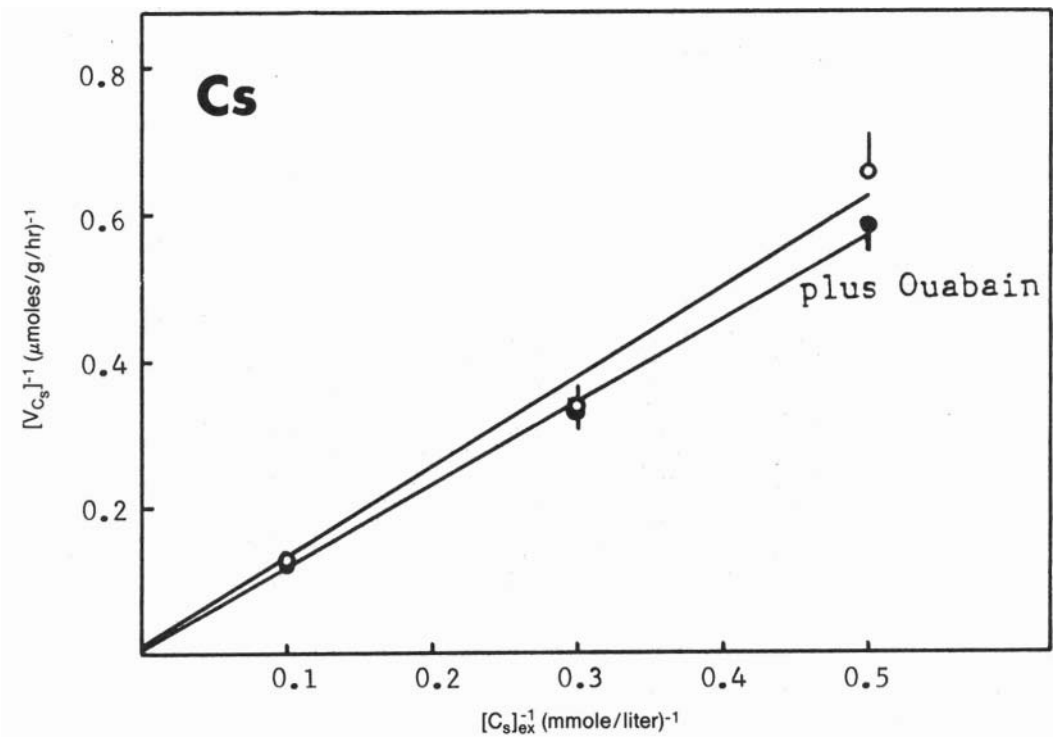

FIGURE 7. Effect of ouabain (10^{-6} M) on the effectiveness of 80 mM Cs in inhibiting labelled Cs^+ entry rate into frog sartorius muscles. Each point represents the average of three determinations. The length of the vertical line above or below the data points is equal to 1 S.E.

of Ludwig Edelmann (1973), then clearly ouabain should also exercise an EDC effect on the β- and γ-carboxyl group mediating alkali-metal ion entry into the cells. Indeed this is the essence of the finding of this report.

Next we would like to mention that if ouabain derives its pharmacological effect primarily by inhibiting the postulated pump, ouabain should fall into the category of a poison and not that of a drug. Yet ouabain is a bonafide member of the class of extremely useful drugs in combating heart failure. Indeed when foxgloves were used in the eighteenth century, it was to combat dropsy (edema). In the conventional membrane pump theory the Na pump is supposed to keep the cell from swelling or becoming edematous by constant outward pumping of Na^+ (for review see McKnight and Leaf, 1977). In this case, a drug that poisons the Na pump, which keeps the cells from becoming edematous, could hardly be expected to combat dropsy.

One asks "What then is the true phamacodynamic reason that ouabain does combat heart failure?" Goodman and Gilman in their classical treatise "The Pharmacological Basis of Therapeutics" (Goodman and Gilman, 1960) pointed out that "The main pharmacological property of digitalis is its unique ability to increase the force of myocardial contraction"(p. 672). Indeed in 1855 Vulpian first demonstrated that digitalis caused a systolic contracture of frog heart (see Goodman and Gilman, 1960, p. 672).

As far back as 1952 Ling has suggested

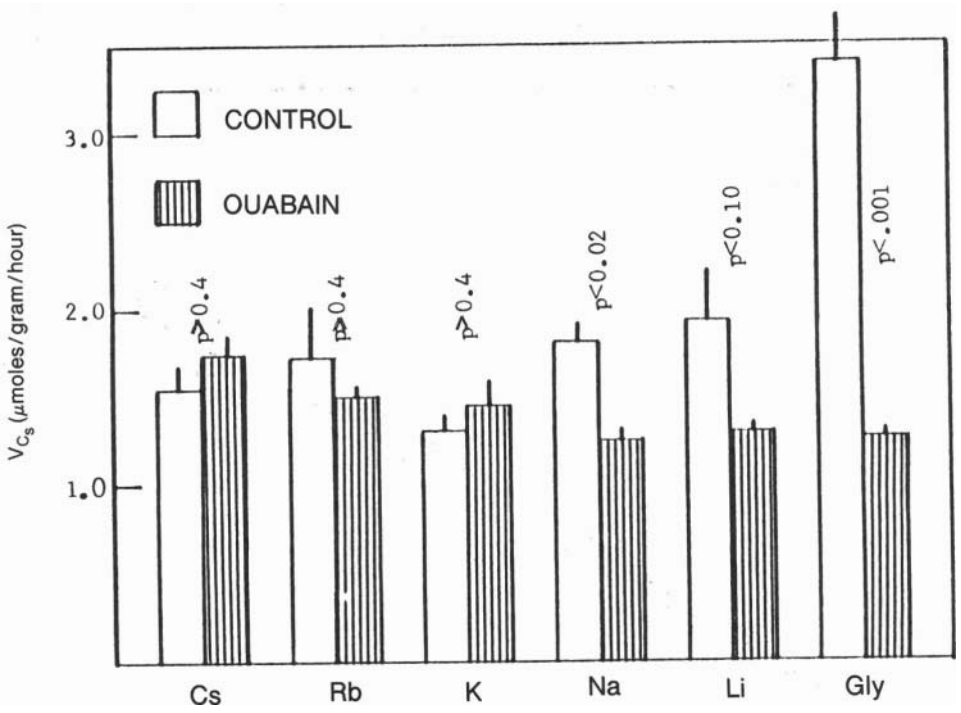

FIGURE 8. The rates of labelled Cs^+ entry into frog sartorius muscle in solution containing 1.89 mM labelled Cs^+ and 80 mM of CsCl, RbCl, KCl, NaCl, LiCl, or glycine. In one series marked with ▭, the muscles were preincubated 4 hrs. in 10^{-6} M ouabain before incubation in the labelled solution also containing 10^{-6} M ouabain. In the control series, marked as □, no ouabain was added to either preincubation or incubation solution. Each value was obtained from 4 determinations on 4 muscles in 2 sets of experiments (gly, Li, Rb, Cs) or 8 determinations on 8 muscles in 4 sets of experiments (Na, K). A highly similar full set of data was obtained at the external labelled Cs^+ concentration of 9.85 mM.

that the rigidity seen in muscle contraction reflects the formation of new "salt linkages" between negatively-charged fixed carboxyl groups and positively-charged fixed amino groups on the contractile proteins (Ling, 1952).

The concept of salt linkages is now established beyond doubt by the exact structural analyses of crystalline proteins (see Perutz, 1969) and by the recent Na^+ binding studies of bovine hemoglobin (Ling and Zhang, 1984). Perutz's study shows that in hemoglobin, salt linkages were formed between cationic ϵ-amino groups and a-amino groups and between anionic γ-carboxyl groups and a-carboxyl groups of hemoglobin (Perutz, 1969).

It has long been known that in various cells studied, K' inhibits glycine uptake and that glycine when added to the external medium reduces K' level in the cell (Christensen and Riggs, 1952). Now that the adsorption of virtually all of the cells K' on fixed carboxyl groups has been proven, a reasonable assumption is that K' and the cationic a-amino group of glycine compete for the same anionic carboxyl groups of cell proteins, an assumption that would also explain why glycine competitively inhibits Cs' entry (Figure 2). With this assumption, the ouabain-induced increase of glycine binding onto the cell surface carboxyl groups demonstrated here may offer a clue to the molecular mechanism underlying the main pharmacodynamic action of the cardiac glycoside. That is, the cardiac glycoside enhances the strength and number of salt linkages formed between fixed carboxyl groups and fixed cationic groups of the contractile proteins during systole of the contracting heart, much as it enhances the binding of the cationic a-amino group of glycine on cell surface anions as demonstrated in Figure 2 of this communication.

SUMMARY

The effects of ouabain on the effectiveness of glycine, Li^+, Na^+, K', Rb^+, and Cs^+ in the external medium in reducing the rate of entry of labeled Cs' into frog sartorius muscles were studied. The results showed that in the absence of ouabain the effectiveness of glycine and alkali-metal ions in inhibiting labeled Cs^+ entry follows the rank order: K' > Cs', Rb^+ > Na', Li' > glycine. Exposure to ouabain in essence reverses this order which then becomes: glycine > Li^+, Na^+ > K', Rb^+ > Cs^+.

These results confirm the prediction of the basic electronic interpretation of drug action according to the association-induction hypothesis. In addition, it shows that the action of ouabain on the surface β- and γ-carboxyl groups of frog muscle mediating Cs' entry is quite similar to its action on the cytoplasmic β- and γ-carboxyl groups that are the seats of K' accumulation in the bulk phase cytoplasm as well as to its action on the cell surface β- and γ-carboxyl groups responsible for the generation of the resting potential. In all these cases, ouabain acts as an electron-donating cardinal adsorbent (EDC). Finally the marked increase of the binding strength of glycine on the surface β- and γ-carboxyl groups was used to explain the primary pharmacodynamic effect of cardiac glycosides in combating heart failure.

The work was primarily supported by Office of Naval Research Contract No. N00014-85-K-0573 and to a lesser extent by NIH Grant 2-R01-CA16301.

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Reviewed August 12, 1987