Extrarrenal effects of diuretic drugs: A basis for the design of new ion transport inhibitors

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Introduction

Diuretic drugs are able to decrease renal Na^+ reabsorption by inhibiting one or more Na^+ transport carriers in different segments of the nephron (fig. 1 and ref. 1). Furosemide and bumetanide provoke natriuresis by inhibition of a [Na^+, K^+, Cl^-]-cotransport system in the luminal side of the Henle's loop. Amiloride inhibits a Na^+ channel located apically in distal tubular cells. Xipamide appears to inhibit HCO_3^--dependent Na^+ reabsorption all along the nephron. Spironolactone antagonizes the aldosterone-induced synthesis of a Na^+-transport protein (via its active metabolite, canrenone) in distal and collecting tubules. The injection of ouabain directly into the renal artery inhibits basolateral Na^+ reabsorption which is catalyzed by the [Na^+, K^+]-pump. Acetazolamide acts like a carbonic anhydrase inhibitor in proximal tubular cells, thus decreasing net $NaHCO_3$ reabsorption. The mechanism of action of thiazide drugs is unclear.

Besides the kidney, all other organs have cells with Na^+ transport systems sensitive to diuretic drugs. This explain (at least in part) the extra-renal actions of diuretic drugs [1]. Molecular studies of the renal and extrarenal actions of diuretic drugs have strongly contributed to our knowledge of membrane ion transport. In particular, the study of membrane ion transport was greatly facilitated by the discovery of potent and quite specific inhibitory drugs. Indeed, the first generation of transport inhibitors was readily discovered because these compounds were therapeutic agents acting on basic transport mechanisms, like the renal Na^+ transport systems (the diuretics described in figure 1) or the myocardial [Na^+, K^+]-pump (the cardiac glycosides).

Erythrocites and other cell systems were used for screening large numbers of diuretics and other compounds on ion transport systems. This has recently enriched the spectrum of transport systems able to be studied by using transport inhibitors. In particular, we have now potent (and more or less specific) inhibitors for the [K^+, Cl^-]-cotransport system, the [Na^+: H^+] exchanger, the [Na^+: Ca^{2+}] exchanger and other ion transport systems (see below). These new compounds are actually submitted to pharmacological and therapeutical studies (looking for immunosupresive or inotropic activity or by an action against brain edema or other disorders of ion metabolism).

Pharmacological studies with the new ion transport inhibitors need to take into account the fact that ion transport systms have isoenzymes with different drug sensitivity (as already demonstrated for the cardiocyte [Na^+, K^+]-pump, which is more sensitive to ouabain than that of kidney cells). Therefore, the relative affinities of compounds for different ion transport systems may be an important feature to consider in structure design, particularly for the tissue specificity of the therapeutic agents.

Use of Human Red Blood Cells for Pharmacological Studies of Ion Transport

The human red blood cell is one of the best models for screening drugs on ion transport mechanisms because: (i) generally speaking, the ion transport systems (or isoenzymes) of human erythrocytes are pharmacologically similar to those of other cells, (ii) the screening of compounds is more rapid (and precise) in erythrocytes than in most in-vivo or ex-vivo assays (for instance in the field of diuretic drugs), (iii) the compounds can be tested in a human cell overcoming hazardous pharmacological extrapolations from animal data, (iv) red cells can be easily obtained in great amounts and free from other contaminating cells, (v) human erythrocytes have only one internal compartment for ions, i.e., the rate of ion exchange between cells and environment can be equated to the ion movements across the plasma membrane, (vi)

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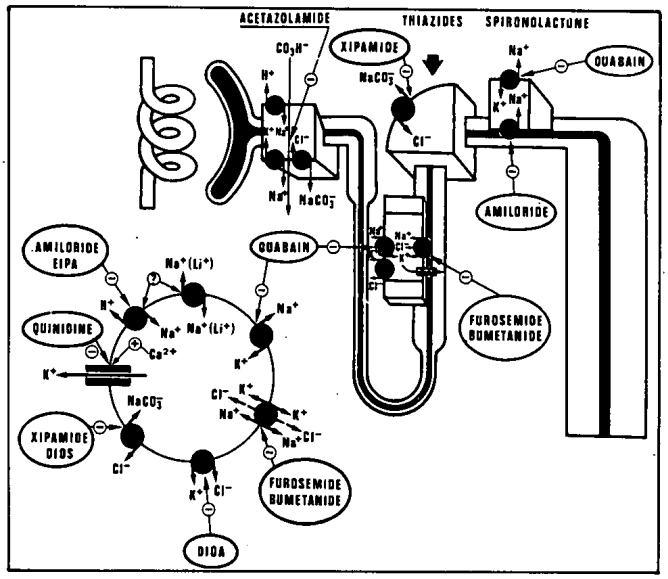

Fig. 1.—Interaction of diuretic and related compounds with Na^+ and K^+ transport systems in the kidney and in human red cells.

cell ion content can be controlled allowing the measurement of ion fluxes under steady-state and initial rate conditions and (vii) correlations with chemical or physical properties of the cell membrane are facilitated by the great amount of information accumulated in the past decades concerning structural and functional properties of the red cell membrane.

It is important to note that some ion transport systems of epithelial or excitable cells are lacking in human red cell membranes and that the existing transport mechanisms catalyze much more important fluxes in other cells (with one exception, the anion carrier; see later). The human red blood cell appears therefore to be a useful but incomplete model for ion transport studies.

Ion Transport Inhibitors

Generally speaking, the transport inhibitors can be divided into three groups:

1. Drugs acting at anion-translocating sites, like furosemide and other *organic acids.*
2. Drugs acting at cation-translocating sites, like amiloride and other *organic bases.*
3. Drugs acting at non-translocating (regulatory) sites, like ouabain and other cardiac glycosides.

Drugs acting at anion-translocating sites

The classical representatives of drugs acting at anion-translocating sites are loop diuretics of the sulfamoylbenzoic acid family (furosemide, bumetanide and piretanide) and those of the (aryloxy)acetic acid family (ethacrynic acid, indacrinone). The common characteristic of these loop diuretics is to be *carboxylic acids.* Their mechanism of action involves a competition of the $R\text{-}COO^-$ group with chloride for a common anionic-receptor site on different Cl^- transport systems[2]. Three of these Cl^- transport systems exist in human erythrocytes:

1. The $[Na^+, K^+, Cl^-]$-cotransport system (for review see ref. 3), which shares similar properties with the cotransport system catalyzing luminal Na^+ reabsorption in the Henle's loop. Indeed good correlations were found between saliuretic activity of lopp diuretics and inhibition of $[Na^+, K^+, Cl^-]$-cotransport system in erythrocytes.
2. The $[Cl^-/HCO_3^-]$ exchange or anion carrier[4, 5]. Loop diuretics are able to inhibit this transport system with IC_{50} of 10^{-4} M[5]. For bumetanide (but not for furosemide or ethacrynic acid), these are 2-3 orders of magnitude higher concentrations than those required to inhibit the $[Na^+, K^+, Cl^-]$-cotransport system. Therefore, bumetanide is a more selective cotransport inhibitor than furosemide.

The classical inhibitors of the anion carrier are the disulfonic stilbenes DIDS and SITS (IC_{50} of 10^{-7}, 10^{-6} M). Their mechanism of action involves a covalent binding with the amino group of a lysine (sulfonamide formation). However, this lysine does not appear to be implicated in transport itself[6] and in the inhibition by diuretics furosemid and ethacrynic acid.

Whether the inhibition of the anion carrier is correlated with a pharmacological action is still unclear. On the other hand, Kimelberg and Bourke et al. have found that astrocyte swelling (a major cell phenomena in cytotoxic brain edema) involves a coupled chloride and cation transport, through a SITS-sensitive, anion-exchange system, associated with the transfer of an osmotic equivalent of water[7]. In addition, loop diuretics, including furosemide and ethacrynic acid, are useful agents for the treatment of brain edema (see 5 for references). Finally, we recently found that new anti-brain edema agents, which are devoid of both diuretic activity and inhibitory ability against the $[Na^+, K^+, Cl^-]$-cotransport system have higher inhibitory activity against the anion carrier than loop diuretics[5]. We supposed that the link between these observations was the ability of anti-brain edema agents (and loop diuretics) to inhibit net $NaCO_3^-$ influx through the astrocyte anion carrier[5]. However, recent results suggest that HCO_3^- ions may stimulate more than one pathway for net Na^+ uptake in astrocytes (P. Hannaert, personal communication). Further investiation is required in order to clarify the pharmacological implications of the anion carrier inhibition.

3. The $[K^+, Cl^-]$-cotransport system[8]. This is a third ion transport system sensitive to loop diuretics (although at concentrations higher than 10^{-3} M,

ref. 8). We have recently found a new family of compounds, [(Dihydroindenyl)oxy]alkanoic acids, which are the first potent inhibitors of the [K^+, Cl^-]-cotransport system without side effects on the [Na^+, K^+, Cl^-]-cotransport system. By using the leading compound of this series, namely DIOA, we have clearly demonstrated the existence of the [K^+, Cl^-]-cotransport system in human red blood cells[8]. The physiological role of this transport system is to catalyze a regulatory volume decrease (RVD) in swollen red cells[8].

DIOA is now being introduced for studying the [K^+, Cl^-]-cotransport system in hematological diseases. It appears that the [K^+, Cl^-]-cotransport system can be: (i) secondarily hyperactivated in stomacytosis[9] and primarily increased in sickle and CC cells[10-13]. The secondarily hyperactivation helps the red cells to counterbalance the increase in cell volume. Conversely, the abnormal activation in sickle (and CC) cells seems to be responsible for the next KCl extrusion and sickle cell dehydration. Interestingly, DIOA inhibited the abnormal sickle cell K^+ loss and specifically reduced sickle cell density upon stimulation of the net outward [K^+, Cl^-]-cotransport by low pH, hypoosmolarity, and NEM (n-ethylmaleimide). Therefore, DIOA may open a new therapeutic approach of sickle cell disease by inhibition of cell dehydration which favors HbS polymerization and reduces red cell deformability.

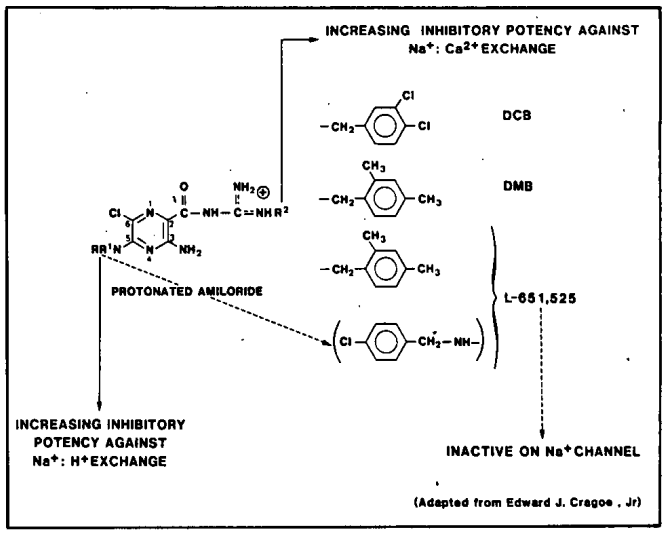

Fig. 2.—Amiloride analogs: Structural requirements for inhibition of [Na^+:Ca^{2+}] or [Na^+:H^+] exchangers.

Drugs acting at cation-translocating sites

Amiloride is one main representative of this group of transport inhibitors (see figure 2). This compound has a guanidine group which is positively charged at physiological pH and can interact with the Na^+ sites of epithelial Na^+ channels, [Na^+:H^+] exchange and some other Na^+ carriers.

In these last years several amiloride analogues have been synthesized in order to increase potency and/or selectivity against the different Na^+ carriers[14, 15]. Figure 2 shows that, generally speaking aromatic residues in N_5 increase the inhibitory potency against [Na^+:H^+] exchange and aromatic residues in the guanidine group increase inhibitory activity against [Na^+:Ca^{2+}] exchange.

The recent discovery of [Na^+:H^+] exchange in human red cells[16] now allows to test different amiloride-analogues in this cell model.

Among the other compounds acting at cation translocating sites we may mention ion (K^+, Na^+ and Ca^{2+}) channel blockers like quinidine, a classical inhibitor of Ca^{2+}-sensitive K^+-channels. However, it is important to note that quinidine may also non-specifically interact with other (non-translocating) sites of different ion carriers.

We have recently screened several compounds on the erythrocyte [Na^+:Mg^{2+}] exchanger[17]. The guiding strategy was based on the screening of organic bases potentially active at the Na^+ site of the transporter. We have found three families of active compounds: cinchona alkaloids, amiloride and tricyclic antidepressant drugs. The members of the latter series (particularly imipramine) seem more specific inhibitors of the [Na^+:Mg^{2+}] exchanger[17].

Drugs acting at non-translocating (regulatory) sites

Cardiac glycosides, the most potent and selective inhibitors of the Na^+, K^+-pump, are representative of drugs acting at regulatory sites.

Pharmacological studies of digitalis drugs were mainly carried out in heart cell membranes (with the remarkable exception of the pump — inhibitory ability of ouabain, originally found in human red cells[18]). These studies have identified several interacting groups or domains in both the cardiac glycoside molecule and the external aspect of the Na^+, K^+-pump[19, 20]. Figure 3 shows a model for ouabain where: (i) a pump-region DY binds the lactone ring by means of a dipolar interaction and (ii) a pump-domain DX interacts through hydrogen bonds with the -OH in C_{14} and with the -OH of the glycoside residue. It is important to note that the hydrophobic reaction of the cyclopentanoperhydrophenantrene ring (not shown in figure 3) plays a principal role in the complex formation of steroid molecules[21]. The digitalis-site seems to be close to the ionophoric pore through which K^+ ions enter into the pump molecule. The model assumes that ouabain-binding inhibits the consecutive opening and closing of this pore that takes place during a pump-cycle (figure 3).

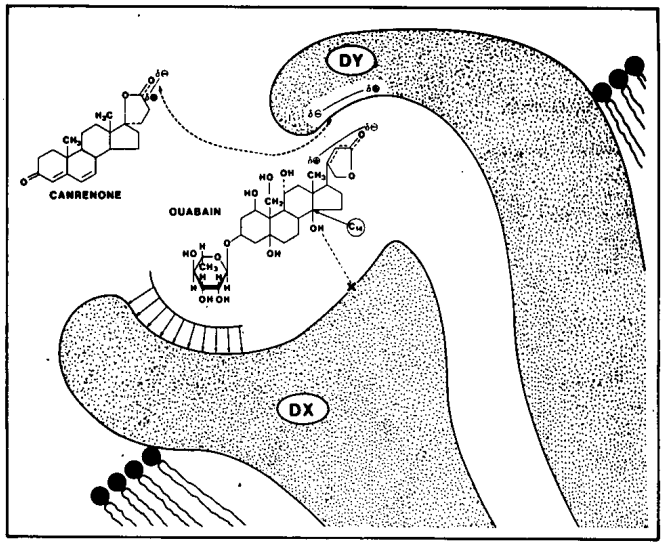

Fig. 3.—A model for the interaction of canrenone at the digitalis receptor site. The regulatory ouabain-site is close to the ionophoric pore for external K^+. During a pump cycle, a conformational transition modifies the distance between domains DY and DX. Canrenone only binds to DY and thus antagonizes ouabain-binding without affecting the conformational transition linked to cation translocation.

The human red cell has been used for the study of potential antagonists of endogeneous "ouabain-like" factors (EOLF). Interest in these compounds was raised by the finding that EOLF were increased in some forms of primary hypertension [22]. Therefore, we postulated that drugs "protecting" the vascular wall against pump-inhibition are of potential interest as a new approach to the treatment of high blood pressure [23]. Canrenone, an antihypertensive drug (see structure in figure 3), exhibited some of the properties required for drugs acting through this mechanism [23, 24].

Canrenone is the active metabolite of spironolactone. Its action mechanism was supposed to involve a competition with aldosterone for a common cytosolic receptor in distal and collecting tubules of the nephron. However, in addition to this mechanism canrenone is able to act like a partial agonist at the digitalis receptor site:

1. Under basal conditions, canrenone slightly inhibits Na^+, K^+-pump activity. An effect which can explain the previous observation that canrenone administration potentiates the inotropic and pressor effects of digitalis drugs [25, 26].
2. If the pump is blocked by high doses of ouabain, canrenone is able to restimulate it. This effect is likely correlated with the ability of canrenone to protect against digitalis-induced cardiac toxicity [27].

In the model of figure 3, canrenone is able to bind to domain DY but not to domain DX. Therefore it antagonizes ouabain binding without hampering the consecutive opening and closing of the K^+ pore.

It is not clear for the moment if chronic administration of canrenone to subjects with primary hypertension may induce a lowering of blood pressure by antagonism with EOLF at the vascular wall. Indeed, in rats with reduced renal mass (under excess Na^+ intake), canrenone is able to partially antagonize *in vivo* the secondary effects of EOLF on cell Na^+-handling and blood pressure [24]. However, chronic treatment with canrenone potentiated (instead of decreasing) the acute pressor effect of ouabain in essential hypertensive patients [26]. Further investigation is required in order to clarify whether canrenone may antagonize EOLF *in vivo*.

Conclusion

The use of some therapeutic agents (like furosemide, amiloride and ouabain) have contributed enormously to the knowledge of membrane ion transport mechanisms. The assay of compounds on such transport systems (particularly in human red cells) is now permitting the development of potent and quite specific transport inhibitors. These new compounds are actually studied at the pharmacological level. This could lead in the near future to the discovery of new therapeutical agents, active in disorders of ion metabolism.

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References

1. Cragoe EJ Jr: In *Diuretics: Chemistry, Pharmacology and Medicine*. p. 174. Ed. by EJ Gragoe Jr. John Wiley. New York, 1983.
2. Haas M and McManus TJ: Bumetanide inhibits (Na + K + 2Cl) co-transport at a chloride site. *Am J Physiol* 245:C235-C240, 1983.
3. Chipperfield AR: The (Na^+, K^+, Cl^-) co-transport system. *Cli Sci* 71:465-476, 1986.
4. Motais R and Cousin JL: In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*. p. 219.Ed by RW Straub and L Bolis. Raven Press, New York, 1978.
5. Garay RP, Hannaert PA, Nazaret C and Cragoe EJ Jr: The significance of the relative effects of loop diuretics and anti-brain edema agents on the Na^+, K^+, Cl^- cotransport system

and the $Cl^-/NaCO_3^-$ anion exchanger. *Naunyn-Schmiedeberg's Arch Pharmnacol* 334:202-209, 1986.
6. Jennings ML: Kinetics and mechanism of anion transport in red blood cells. *Annu Rev Physiol* 47:519-533, 1985.
7. Bourke RS, Kimelberg HK, Daze M and Church G: Swelling and ion uptake in cat cerebrocortical slices: Control by neurotransmitters and ion transport mechanisms. *Neurochem Res* 8:5-24, 1983.
8. Garay RP, Nazaret C, Hannaert PA and Cragoe EJ Jr: Demonstration of a [K^+, Cl^-]-cotransport system in human red cells by its sensitivity to [(Dihydroindenyl)oxy]alkanoic acids: regulation of cell swelling and distinction from the bumetanide-sensitive [Na^+, K^+, Cl^-]-cotransport system. *Mol Pharmacol* 33:696-701, 1988.
9. Morlé L, Pothier B, Alloisio N, Féo C, Garay R, Bost M and Delaunay J: Reduction of membrane band 7 and activation of volume-stimulated [K^+, Cl^-]-cotransport in a case of congenital stomatocytosis. *British J Hematol* 71:141-146, 1989.
10. Vitoux D, Olivieri O, Garay RP, Cragoe EJ Jr, Galacteros F and Beuzard Y: Inhibition of K^+ efflux and dehydration of sickle cells by DIOA: an inhibitor of the [K^+, Cl^-]-cotransport system. *Proc Natl Acad Sci* 1989 (in press).
11. Brugnara C, Kopin AK, Bunn HF and Tosteson DC: Regulation of cation content and cell volume in erythrocytes from patients with homozygous hemoglobin C disease. *J Clin Invest* 75:1608-1617, 1985.
12. Brugnara C, Bunn HF and Tosteson DC: Regulation of erythrocyte cation and water content in sickle cell anemia. *Science* 232:388-390, 1986.
13. Canessa M, Fabry ME, Blumenfeld N and Nagel RL: Volume-stimulated, Cl^--dependent K^+ efflux is highly expressed in young human red cells containing normal hemoglobin or HbS. *J Membrane Biol* 97:97-108, 1987.
14. Kaczorowski GJ, Barros F, Dethmers JK, Trumble MJ and Cragoe EJ Jr: Inhibition of Na/Ca exchange in pituitary plasma membrane vesicles by analogues of amiloride. *Biochemistry* 1985:1394-1403, 1985.
15. Zhuang Y, Cragoe EJ Jr, Shaikewitz T, Glaser L and Cassel D: Characterization of potent Na^+/H^+ inhibitors from the amiloride series in A431 cells. *Biochemistry* 23:4481-4488, 1984.
16. Escobales N and Canessa M: Amiloride-sensitive Na transport in human red cells: evidence for a Na/H exchange system. *J Memb Biol* 90:21-28, 1986.
17. Féray JC and Garay RP: Demonstration of a Na^+:Mg^{2+} exchange in human red cells by its sensitivity to tricyclic antidepressant drugs. *Naunyn-Schmiedeberg's Arch Pharmacol* 338:332-337, 1988.
18. Schatzmann HJ: Herzglycoside als Hemstoff für den aktiven Kalium and Natrium transport durch die Erythrocyten Membran. *Helv Physiol Pharmac Acta* 11:346-354, 1953.
19. Thomas R, Allen JC, Pitts BJR and Schwartz A: Cardenolide analogues. An explanation for the unusual properties of AY 22241. *Eur J Pharmacol* 53:227-237, 1979.
20. Schwartz A, Lindenmayer GE and Allen JC: The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects. *Pharmacol Rev* 27:3-134. 1975.
21. Ioffe DV: Sterols as complex-forming species. *Russian Chem Rev* 55:143-152, 1986.
22. DeWardener HE and MacGregor GA: In *Hypertension*. p. 84. Ed by J General, Okuchel, P Hamet and M Cantin. McGraw Hill, New York, 1983.
23. Garay RP, Díez J, Nazaret C, Dagher G and Abitbol JP: The interaction of canrenone with the Na^+, K^+ pump in human red blood cells. *Naunyn-Schmiedeberg's Arch Pharmacol* 329:311-315, 1985.
24. DeMendonça M, Grichois ML, Pernollet MG, Wauquier I, Trouillet-Thormann B, Meyer P, Devynck MA and Garay RP: Antihypertensive effect of canrenone in a model where endogenous ouabain-like factors are present. *J Cardiovascular Pharmacol* (in press).
25. Marchetti G, Vitolo E, DiFrancesco GF, Cavallaro B and Sponzilli C: Positive inotropic effect of K-Canrenoate: an investigation in anaesthetized dogs untreated or treated with digitalis. *Arch Int Pharmacodyn Ther* 266:250-263, 1983.
26. Laurent S, Hannaert PA, Girerd M, Safar M and Garay R: Chronic treatment with canrenone potentiates the acute pressor effects of ouabain in essential hypertensive patients. *J of Hypertension* (in press).
27. Yeh BK, Chiang BN and Sung PK: Antiarrhythmic activity of K-Canrenoate in man. *Am Heart J* 92:308-314, 1976.