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Entry and Distribution of Chlorpromazine and Vinblastine into Human Erythrocytes during Endocytosis[1] (41252)

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Abstract. Drug-induced endocytosis in intact human erythrocytes is a membrane-mediated event involving membrane invagination and fusion. The drugs capable of inducing endocytosis are amphipathic cations and it was thought that they intercalate themselves into the relatively electronegative cytosolic portion of the phospholipid bilayer. The current studies show that at concentrations that produce endocytosis vinblastine and chlorpromazine are not only concentrated in the red cell membrane compartment but reach high levels in the cytosol as well. The suspending medium can produce impressive effects on the extent of endocytosis. Sodium lactate enhances chlorpromazine and vinblastine endocytosis without altering the incorporation or distribution of these agents. Sucrose enhances chlorpromazine and inhibits vinblastine endocytosis, and while it produces no effects on chlorpromazine incorporation it inhibits vinblastine incorporation, thus in part accounting for its effects. It is proposed that the amphipathic drugs that cause endocytosis produce both membrane and cytosolic alterations which interact to result in the membrane internalization.

The phenomenon of drug-induced endocytosis in intact human RBC offers an opportunity to study red cell membrane-mediated events (1, 2). At least four classes of drugs represented by primaquine, vinblastine, chlorpromazine, and hydrocortisone can induce RBC endocytosis. Contrary to our initial speculations (1), it appears that the mechanism by which endocytosis is produced differs for each agent. For example, primaquine endocytosis is absolutely dependent on provision of energy by ATP (1, 4) whereas chlorpromazine and vinblastine induced endocytosis can take place in metabolically depleted RBC containing virtually no detectable ATP. When Ca^{2+} is introduced into RBC, primaquine endocytosis is stimulated and vinblastine endocytosis is inhibited (4). Incubation of RBC in a nonpenetrable medium like isotonic sucrose or sodium glutamate inhibits primaquine and vinblastine endocytosis but causes an eight- to ninefold increase in hydrocortisone endocytosis (3). The substitution of anions like lactate and acetate for chloride causes a two- to threefold enhancement in primaquine endocytosis. One possible explanation for these differences is that the endocytosis-inducing drugs are metabolized differently by human erythrocytes. In fact, more pamaquine (an easy to analyze analog of primaquine) enters red cells in the presence of sodium lactate than in the presence of saline, thus, in part explaining the enhancing effect of sodium lactate on primaquine endocytosis (2, 4). Therefore, we studied the entry and distribution of ^{3}H-labeled vinblastine and chlorpromazine into human erythrocytes during endocytosis.

In RBC, chlorpromazine is known to produce stomatocytic shape changes (5), to inhibit Ca^{2+} efflux (6) and its linked Ca^{2+}, Mg^{2+}-ATPase (6, 7), to inhibit red cell ghost endocytosis (7), to expand the red cell membrane thereby protecting against osmotic hemolysis (8), and to block chloride self-exchange and glucose permeability (9). Chlorpromazine uptake into red cells has been studied spectrophotometrically measuring the disappearance of chlorpromazine from the suspending medium (10), however, no kinetic analysis was attempted and the method used did not allow for the investigators to determine whether the chlorpromazine was predominantly located in cytosol or membrane compartments. When ghost membranes were incu-

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bated with radioactive chlorpromazine, there was a striking partition of drug into membranes (8), and it was subsequently proposed that the cationic phenothiazines produced stomatocytosis by inserting themselves into and expanding the cytosolic phosphatidylserine- and phosphatidylethanolamine-enriched portion of the lipid bilayer (11). More recently, when intact human RBC were incubated with [^{3}H]chlorpromazine using hygroscopic desorption, a method which does not depend on centrifugation to separate RBC from suspending supernatant, very little membrane binding occurred (12, 13). However, when the same methodology was used at somewhat lower concentrations of [^{3}H]chlorpromazine, binding to intact RBC and ghosts was observed (14). Therefore, the extent of RBC membrane localization of [^{3}H]chlorpromazine is in contention. Vinca alkaloids interact with microtubules thereby interfering with the mitotic spindle, cellular motility (15), and membrane receptor mobility (16). However, little is known about the incorporation and distribution of vinblastine in human erythrocytes. In view of the fact that most studies using [^{3}H]chlorpromazine have shown localization in the membrane, it was anticipated that both of the endocytic agents used would show substantial binding to the red cell membrane.

Materials and Methods. *Materials.* [^{3}H]Chlorpromazine hydrochloride, 21 Ci/mmole, was obtained from New England Nuclear, and [^{3}H]vinblastine sulfate, 11.3 Ci/mmole, was obtained from Amersham. Unlabeled chlorpromazine was obtained as a generous gift from the Eli Lilly Company and unlabeled vinblastine was purchased from Eli Lilly Pharmaceutical Company. [^{3}H]Chlorpromazine was added to stock solutions of unlabeled chlorpromazine in order to produce specific activities ranging from 475 to 965 cpm/nmole. [^{3}H]Vinblastine was added to stock solutions of unlabeled vinblastine to produce specific activities of 290 to 1200 cpm/nmole. Protosol and Biofluor were obtained from New England Nuclear.

Methods. Freshly drawn venous blood was obtained from normal human volunteers according to a protocol approved by the Stanford Committee on Human Experimentation. Erythrocytes were heparinized and then washed once with saline and three times with Hanks' solution.

Drug-induced endocytosis was measured by phase microscopy and by our quantitative radioisotopic method (1, 2). Briefly, in the radioisotopic method the outer surface of the RBC membrane is labeled with a complex of [^{57}Co]vitamin B_{12} and the vitamin B_{12} binding proteins. After endocytosis is induced, the red cells are extensively washed and lightly trypsinized, a procedure that removes over 99% of outer membrane bound [^{57}Co]vitamin B_{12} complex. However, [^{57}Co]vitamin B_{12} complex trapped in endocytic vacuoles is not accessible to washing or trypsinization and the residual ^{57}Co radioactivity provides an accurate measurement of endocytosis (1, 2, 4). We regularly measure drug-induced endocytosis at 37° in a reaction mixture of 1 vol of Hanks' washed packed RBC, 1 vol of Hanks' solution, and 1 vol of plasma, adding the drug at the beginning of the 37° incubation and removing duplicate 0.7-ml aliquots at designated intervals for microhematocrit and radioisotopic determination in an auto γ counter.

In measuring endocytosis in isotonic sodium lactate or isotonic sucrose, the last wash of the RBC is done in either sodium lactate or sucrose, and 1 vol of the washed packed RBC is added to 1 vol of either sodium lactate or sucrose and 1 vol of plasma that had been extensively dialyzed against either sodium lactate or isotonic sucrose supplemented as described (2) with 5 mM Ca^{2+} and 2 mg glucose/ml.

Since our interest is in vinblastine and chlorpromazine endocytosis the experiments on the entry and distribution of [^{3}H]-vinblastine and [^{3}H]chlorpromazine into RBC were designed to parallel the conditions under which endocytosis is routinely studied. The reaction mixture usually contains 1/3 of plasma (see above), which could bind vinblastine or chlorpromazine thereby altering the concentration of free drug. Screening experiments were therefore performed using [^{3}H]chlorpromazine and

[^{3}H]vinblastine in which the entry and distribution was measured in the presence or absence of plasma. There were no differences, therefore, the studies to be reported were performed in plasma-free media and the reactions consisted of 1 vol of Hanks' (or sodium lactate or sucrose) washed packed RBC and 2 vol of either Hanks' solution or isotonic sodium lactate or isotonic sucrose. The reaction mixtures were preincubated in a shaking water bath for 5 min at 37° to bring the contents to temperature, at which point the reaction was started by drug addition. Duplicate 0.7-ml aliquots were removed at the designated times; the microhematocrit was determined and was generally between 15 and 25. The 0.7-ml aliquots were then added to 10 ml of ice-cold phosphate-buffered saline (PBS) to stop the reaction. The mixture was then centrifuged at 12,000*g* in a Sorvall refrigerated centrifuge RC-5 for 5 min. The total supernatant was aspirated, and the volume was measured. The pelleted RBC were brought to 1 ml by careful addition of PBS. In order to establish a baseline for observing the uptake of [^{3}H]vinblastine and [^{3}H]-chlorpromazine into red cells, a "0" time sample was obtained. When the washed RBC and buffer had been preincubated (without drug) for 5 min at 37°, prewarmed drug solution was then added, the mixture vortexed for 5 sec, and then duplicate aliquots were immediately removed as described above and placed into a 15-fold volume of ice-cold PBS and immediately centrifuged. Therefore, in the "0" time sample, drug was in contact with RBC at the designated concentrations at 37° for 5+ sec and at 1/15 the concentration at 4° for approximately 5 min. In order to fractionate RBC into a cytosolic and membrane compartment, duplicate aliquots of these RBC, brought to 1 ml with PBS as described, were disrupted by ultrasonication or by shell freezing and thawing twice in dry ice–acetone. Since the results were comparable, only freeze–thaw data are presented. The disrupted RBC were centrifuged at 41,000 rpm (100,000*g*) in the L5-50B Beckman ultracentrifuge for 60 min at 4°. The clear supernatant hemolysate representing cytosol was carefully removed and brought to a volume of 1 ml and analyzed as described above. The residual pellet consisting of membrane and adherent cytosol was thoroughly mixed and brought to 0.1 ml with PBS and the radioisotopic activity was determined.

Radioisotopic determination: In all experiments there was a supernatant and RBC fraction for analysis and in most experiments, the intact RBC were subfractionated into a cytosol and membrane compartment. In determining the radioisotopic activity in each of these four fractions, duplicate 20- and 40-μl aliquots were added to 200 μl of Protosol in ethanol (1:2), and 0.3 ml of 30% H_2O_2 to dissolve the proteins. Then 10 ml of Biofluor and 0.33 ml of 0.5 *N* HCl were added and the radioactivity in the samples was determined in the Packard Liquid scintillation spectrometer.

Results. Incubation of Hanks' washed human RBC in either 0.5 m*M* vinblastine or chlorpromazine of equivalent specific activity produced interesting differences (Table I). For ease of evaluation of the data in Table I, two columns are shown. Column A represents the actual amount of drug in the fraction recalculated for a 1.0-ml sample volume rather than the 0.7-ml sample actually taken. Column B is the concentration of drug in the fraction corrected for plasmacrit or hematocrit. By the time the "0" time sample of RBC had been separated, chlorpromazine entry into erythrocytes was complete, the concentration of chlorpromazine per milliliter packed RBC (uncorrected for RBC water) being approximately 2½ times the equilibrium concentration of 577 nmole/ml. In contrast, vinblastine entry into RBC took place during the first 5 min of incubation at 37° after which no further net RBC uptake occurred. The vinblastine concentration in RBC (uncorrected for RBC water) rose to levels 1½ times the calculated equilibrium value of 573 nmole/ml. The drop in red cell content of chlorpromazine and vinblastine seen at 120 min of incubation presumably occurred because extensive endocytosis leads to considerable hemolysis as previously reported (1, 2).

TABLE I. DRUG ENTRY INTO RED CELLS

	Time of incubation (min) at 37°							
	0		5		15		120	
Drug added	A[a]	B[b]	A	B	A	B	A	B
Vinblastine (573 nmole/ml, sp act 521 cpm/nmole)								
Supernatant	495	660	299	398	344	459	331	442
RBC (Hct 25)	22	87	226	903	209	837	146	584
Recovery	517		525		553		477	
%	90		92		97		83	
Chlorpromazine (577 nmole/ml, sp act 476 cpm/nmole)								
Supernatant	275	328	284	339	302	360	320	381
RBC (Hct 16)	247	1544	214	1339	224	1398	205	1284
Recovery	522		498		526		525	
%	90		86		91		91	

[a] Column A: Actual amount of drug (nanomoles) measured in the indicated fraction recalculated for a 1-ml sample instead of the 0.7-ml sample actually used.

[b] Column B: The concentration of drug in nanomoles/ml of supernatant (corrected for plasmacrit) or nanomoles/ml packed RBC (corrected for hematocrit).

The extent of chlorpromazine and vinblastine endocytosis can be altered by modifying the suspending medium. Isotonic sucrose inhibits vinblastine endocytosis, as previously shown (2), and stimulates chlorpromazine endocytosis (Table II). In contrast, isotonic sodium lactate stimulates both chlorpromazine and vinblastine endocytosis. Therefore, chlorpromazine and vinblastine entry into RBC from these three media were studied. Prior reports had suggested that chlorpromazine localized in the RBC membrane (8, 11) while microtubular analogs that would bind vinblastine might reasonably be found in the red cell membrane. Therefore, RBC were fractionated in order to determine the proportion of vinblastine and chlorpromazine in the membrane and cytosolic compartments. Lower concentrations of chlorpromazine (0.15 m*M*) were used in these experiments to avoid overloading the membrane should it

TABLE II. VINBLASTINE- AND CHLORPROMAZINE-INDUCED ENDOCYTOSIS IN THREE DIFFERENT MEDIA

	Vacuole formation[a]		
	Hanks'	Sucrose	Sodium lactate
Donor 1			
0.6 m*M* chloropromazine	400	1747 (434)[b]	2138 (534)
0.5 m*M* vinblastine	838	469 (56)	1281 (153)
Donor 2			
0.6 m*M* chlorpromazine	763	2229 (292)	2056 (269)
0.5 m*M* vinblastine	994	456 (46)	1225 (123)
Donor 3			
0.6 m*M* chlorpromazine	188	981 (522)	1132 (602)
0.5 m*M* vinblastine	863	606 (70)	1363 (158)

[a] Extent of vacuole formation is recorded as Δ[^{57}Co]vitamin B_{12} trapped, corrected for membrane binding of [^{57}Co]vitamin B_{12}.

[b] Percentage of endocytosis in Hanks' medium is given in parentheses.

have transpired that membrane binding was a prominent feature of chlorpromazine incorporation.

As before (Table I) RBC incorporation of chlorpromazine was essentially complete at the "0" time sample (Table III). Recovery of radioisotopic drug from cytosol and membrane fractions was virtually complete so that the two values, when totalled, regularly equalled the value of radioisotopic drug contained in intact RBC. Therefore, in experiments showing the fractionation of RBC into a membrane-enriched pellet and cytosol only the membrane data are shown in the table. The proportion of chlorpromazine in the membrane fraction varied from experiment to experiment perhaps in part due to individual donor variation. This variability in results obtained with chlorpromazine is in contrast to the results obtained with vinblastine (Table VI). Since the conditions of incubation, separation, fractionation, and radioisotopic analysis were identical for both drugs, the variability suggests that entry of chlorpromazine into RBC is somehow a less regular phenomenon. Nevertheless, the important point is that the paired experimental values for the chlorpromazine content of RBC and their membranes suspended in either Hanks' or sodium lactate medium were generally similar. At this concentration of chlorpromazine, most of the drug (80–90%) appeared in the cytosol.

The proportion of vinblastine in the membrane fraction was quite constant in the face of variation in the concentration of vinblastine added to reactions (Table IV, donors 3A and B). Incubation of RBC in Hanks' or sodium lactate gave comparable results.

Despite the three- to fivefold increase in chlorpromazine endocytosis induced by sucrose (Table II), there was no regular sucrose induced alteration in chlorpromazine incorporation or distribution within RBC (Table V). Sucrose clearly reduced the rate of vinblastine uptake into RBC but not the proportion of vinblastine recovered in the membrane fraction (Table VI). The degree of sucrose-induced inhibition of vinblastine endocytosis (46–70% of control, Table II) is similar to the inhibition of vinblastine uptake (34–77% of control, Table VI) seen at 15 min of incubation.

Discussion. Concentrations of chlorpromazine chosen for this study are higher than those customarily used because we

TABLE III. CHLORPROMAZINE (0.15 m*M*) ENTRY AND DISTRIBUTION INTO RBC SUSPENDED IN HANKS' OR SODIUM LACTATE

	Time of incubation (min) at 37°							
	0		5		30		120	
	Hanks'	Sodium lactate	Hanks'	Sodium lactate	Hanks'	Sodium lactate	Hanks'	Sodium lactate
Donor 1								
Supernatant[a]	137	131			111	115	130	108
RBC[a]	205	215			232	245	228	246
Membrane[b]	13	13			22	14	16	14
Donor 2								
Supernatant[a]	101	96			99	80	92	76
RBC[a]	291	304			287	353	320	379
Membrane[b]	11	11			13	12	23	16
Donor 3								
Supernatant[a]	117	116	118	107	113	112	170	88
RBC[a]	263	261	268	287	266	272	265	335
Membrane[b]	9	3	11	3	12	3	5	9

[a] Chlorpromazine concentration in supernatant solution and RBC expressed as nanomoles/ml exactly as described in Table I, column B.

[b] RBC were fractionated into cytosol and membrane fractions (see Methods) and the amount of CPZ in the membrane pellet is reported as percentage of total RBC content.

TABLE IV. VINBLASTINE ENTRY AND DISTRIBUTION INTO RBC SUSPENDED IN HANKS' OR SODIUM LACTATE

	Time of incubation (min) at 37°							
	0		5		15		120	
	Hanks'	Sodium lactate	Hanks'	Sodium lactate	Hanks'	Sodium lactate	Hanks'	Sodium lactate
Donor 1[a] (VLB concentration, 0.5 m*M*)								
Supernatant[b]	660	664	398	480	459	446	441	527
RBC[b]	96	73	903	833	920	911	659	656
Membrane[c]	5	3	3	3	3	3	3	4
Donor 2 (VLB concentration, 0.5 m*M*)								
Supernatant[b]	610	566	430	328	384	358	376	432
RBC[b]	71	61	863	855	767	831	714	616
Membrane[a]	7	3	3	2	3	1	6	4
Donor 3A (VLB concentration, 0.25 m*M*)								
Supernatant[b]	357		228		228			
RBC[b]	74		336		337			
Membrane[a]	8		3		3			
Donor 3B (VLB concentration, 0.5 m*M*)								
Supernatant[b]	641		453		440			
RBC	124		597		701			
Membrane[c]	7		2		3			

[a] Part of this experiment is recorded in Table I.

[b] Vinblastine concentration in supernatant solution and RBC expressed as nanomoles/ml exactly as described in Table I, column B.

[c] RBC were fractionated into cytosol and membrane fractions (see Methods) and the amount of VLB in the membrane pellet is reported as percentage of total RBC content.

TABLE V. CHLORPROMAZINE (0.15 mM) ENTRY AND DISTRIBUTION INTO RBC SUSPENDED IN HANKS' OR SUCROSE

Time of incubation (min)	Hanks'		Sucrose	
	nmoles CPZ/ml RBC	% CPZ in membrane fraction	nmoles CPZ/ml RBC	% CPZ in membrane fraction
Donor 1				
0	315	2	355	4
120	342	5	393	9
Donor 2				
0	324	3	330	8
120	374	6	365	4
Donor 3				
0	361	6	338	5
120	389	7	402	4
Donor 4				
0	246	21	191	27
120	247	15	263	13
Donor 5				
0	219	21	206	27
120	244	17	296	16
Donor 6				
0	234	19	188	19
120	254	14	268	22

were particularly interested in the conditions of drug entry and distribution as they occur coincident with drug-induced endocytosis. Chlorpromazine is very promptly taken up into red cells (Tables I, III, and V) at these high concentrations. This rapid association of chlorpromazine with red cells is also seen at much lower concentrations (0.1 to 10 μM) (14) where it is thought that very little chlorpromazine enters the cytosol (14). An unexpected finding is that at a concentration of 0.15

TABLE VI. VINBLASTINE (0.5 mM) ENTRY AND DISTRIBUTION INTO RBC SUSPENDED IN HANKS' AND SUCROSE

Time of incubation (min)	Hanks'		Sucrose	
	nmoles VLB/ml RBC	% VLB in membrane fraction	nmole VLB/ml RBC	% VLB in membrane fraction
Donor 1				
15	322	9	249 (77)	10
120	303	11	269 (89)	10
Donor 2				
15	465	7	160 (34)	5
120	317	11	251 (79)	3
Donor 3				
15	459	6	179 (39)	7
120	332	8	258 (78)	8
Donor 4				
15	506	4	370 (73)	5
120	591	5	688 (116)	6
Donor 5				
15	532	4	388 (72)	5
120	532	7	526 (99)	6
Donor 6				
15	682	3	383 (56)	4
120	617	6	662 (107)	6

[a] Percentage of respective value in Hanks' solution.

m*M*, 80–90% of the chlorpromazine in the red cells is in the cytosol with the remaining 10–20% being in the membrane pellet of plasma membranes variably contaminated with adherent cytosol. In terminating the incubation, 0.7-ml aliquots (see Methods) were added to 10 ml of ice-cold PBS and this step served to acutely reduce the temperature and to reduce the supernatant concentration of chlorpromazine to about 26 nmole/ml (275 nmole divided by approximately 10.5 ml, Table I, "0" time). Because the RBC concentration of chlorpromazine was 1544 nmole/ml, there was probably some loss of chlorpromazine from red cells even at 0–4° during the 5 min required for separation. Since the membrane is the interface between the red cell and this lower concentration of chlorpromazine it is probable that as chlorpromazine leaves the red cell, relatively more will come from the membrane compartment. Therefore, the membrane content of drug in these studies is probably underestimated. The finding that most of the chlorpromazine was in the cytosol was surprising but should not detract from the relatively large amount of chlorpromazine that was membrane bound. Since the volume of the membrane is less than 2% of the red cell volume (14), any amount of drug in the membrane which is consistently in excess of 2% of the red cell content represents a high concentration of drug in the membrane. All of our values for chlorpromazine in the membrane compartment are 2% or greater (Tables III and V), thereby indicating an impressive concentration of chlorpromazine in the membrane.

Vinblastine enters red cells more slowly than chlorpromazine (Tables I and IV). Most of the vinblastine is recoverable from the cytosol but 1–11% of RBC vinblastine is in the membrane compartment (Tables IV and VI). Given the fact that membrane drug content is underestimated in these studies and that values for membrane drug content in excess of 2% of RBC drug content represents substantial concentration, it can be argued that most of our values for membrane vinblastine reflect relatively high levels in the membrane (Tables IV and VI). An analog of tubulin has not been identified in human red cells.

Both chlorpromazine and vinblastine reach steady-state levels within RBC which are above the equilibrium concentration. It is likely that chlorpromazine and vinblastine enter RBC by simple diffusion after which the drug is bound to protein and hence, not available for back diffusion. Active transport seems unlikely because both chlorpromazine and vinblastine can produce extensive endocytosis in RBC which have been almost totally depleted of ATP (3, 4).

Sodium lactate enhances both chlorpromazine and vinblastine endocytosis (Table II) but has no effect on the incorporation or distribution of chlorpromazine or vinblastine (Tables III and IV). This observation contrasts with the findings seen with primaquine where lactate enhancement of pamaquine endocytosis is paralleled by an increase in pamaquine incorporation into RBC (4). In isotonic sucrose, a dehydrating medium, the enhancement of chlorpromazine endocytosis (Table II) is not reflected in alterations of chlorpromazine incorporation (Table V). However, the sucrose-induced inhibition of vinblastine endocytosis (Table II) is paralleled by an inhibition of vinblastine incorporation seen in the first 15 min of incubation (Table VI).

It was initially attractive to try to formulate drug-induced endocytosis as a common pathway leading from drug-induced stomatocytosis (5) to endocytic vacuole formation (1). The stomatocytosis seemed explicable on the basis of the postulate that amphipathic cations (all endocytic drugs are amphipathic cations) intercalated into the relatively electronegative cytosolic half of the lipid bilayer, swelling it and causing the inward buckling that leads to stomatocytosis (5, 8, 11). Presumably the stomatocytic state provided a necessary but not sufficient precondition for endocytosis. The further action of the endocytosis-inducing drugs could then have provided the increment that leads to endocytosis. However, while chlorpromazine does achieve relatively high concentrations in the membrane, at the levels of chlorpromazine required to produce endocytosis there is considerable chlorpromazine in the cytosol as well (Tables III and V). The same can be said for vinblastine (Tables IV

and VI) and pamaquine (4). It therefore appears that endocytosis-inducing amphipaths achieve high levels in both the membrane and cytosol. This observation leads to the hypothesis that it is the interaction of drug-induced membrane and cytosolic events that leads to endocytosis. Unfortunately, each drug capable of inducing endocytosis appears to have some unique action. Primaquine action requires ATP and is enhanced by Ca^{2+} addition (4), while vinblastine and chlorpromazine do not require ATP (3, 4). Chlorpromazine is not affected by Ca^{2+} addition while vinblastine is inhibited (4). The role of the suspending medium is also complex as noted above. A very dehydrating medium like sucrose enhances hydrocortisone and chlorpromazine endocytosis but inhibits vinblastine and primaquine endocytosis. Cytosolic chlorpromazine could play a role in endocytosis via its interaction with Ca^{2+} control mechanisms. Ca^{2+} movements are important in drug-induced endocytosis (17, 18) and the chlorpromazine-induced inhibition of Ca^{2+} efflux and its linked Ca^{2+}, Mg^{2+}-ATPase (6) probably occurs via antagonism of the activation of Ca^{2+}, Mg^{2+}-ATPase by cytosolic calmodulin (19). Thus it may be that both membrane and cytosolic events are produced by the amphipaths that have the capacity of inducing endocytosis. The mechanism by which different media, like sodium lactate and isotonic sucrose, affect these interactions remains to be determined.

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