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Absorption-partition relationships for true homologous series of xenobiotics as a possible approach to study mechanisms of surfactants in absorption. III. Aromatic amines and cationic surfactants

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Summary

The previously proposed approaches for interpreting and quantifying the mechanisms elicited by nonionic surfactants on the intestinal and colonic absorption of 4-alkylanilines are extended here to the cationic surfactants dodecyltrimethylammonium bromide and tetradecyltrimethylammonium bromide. The effects of the cationics run parallel to those observed for nonionics and the only modification observed is a more effective membrane polarity increase, possibly inherent to their cationic nature. Therefore, the mechanisms involved in xenobiotic absorption modifications seem to be characteristic of the surfactants and independent of their ionization properties.

Introduction

In preceding papers (Plá-Delfina et al., 1987; Collado et al., 1988), comprehensive relationships were established between the absorption rate constants determined in situ in the rat colon or small intestine in the absence of surfactant, in the presence of polysorbate 80 at its critical micelle concentration (CMC) and at a supramicellar concentration (SMC), and lipophilicity constants were evaluated for 4-alkylanilines in vitro. Through analysis of the equations derived in each case, the modifications observed in the absorption behaviour of the xenobiotics were satisfactorily explained, thus leading to a possible interpretation of the mechanisms underlying the effects of surfactants on the passive absorption of drugs and other xenobiotics.

In the present study, the influence of the cationic surfactants on the same xenobiotics was tested in order to ascertain whether the abovementioned effects and mechanisms are inherent to surfactant substances, i.e. independent of their ionization characteristics.

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Materials and Methods

Xenobiotics and surfactants

The previously tested compounds (aniline and its 4-methyl, 4-ethyl, 4-propyl and 4-butyl derivatives) were used as xenobiotics. For use as surfactants, two cationic amphiphiles were selected: dodecyl- and tetradecyltrimethylammonium bromide (DTAB and TTAB, respectively) for experiments on colon and small intestine.

Absorption technique

The in situ rat gut absorption technique (Doluisio et al., 1969), adapted as described previously (Martín-Villodre et al., 1986) and using the colon and whole small intestine, was performed on male Wistar rats weighing 210–285 g. The preparation procedure and characteristics of the perfusion solutions were the same as those reported earlier (Martín-Villodre et al., 1986; Plá-Delfina et al., 1987; Collado et al., 1988). The xenobiotic solutions were adjusted to pH 7.5 or 6.2 for tests on colon and small intestine, respectively. Five animals per compound and series were utilized.

Two series of absorption experiments were carried out in each case: in the presence of surfactant below its CMC (0.0125% for DTAB and 0.018% for TTAB; both w/v) in the perfusion fluid (yielding k_0 values), and in the presence of the surfactant at a clear SMC (1.0% for DTAB and 0.50% for TTAB, both w/v) in the luminal solution (k_s values). The results obtained were compared among themselves and with those reported previously for the xenobiotics in the absence of surfactant, i.e. in free solution (k_a values) (Martín-Villodre et al., 1986).

Significant reduction in volume of the perfusion fluids at the end of samplings was noted only for the small intestine; therefore, a correction for water reabsorption was made for these data series, according to an earlier method (Martín-Villodre et al., 1986). The remaining concentrations (already corrected for the small intestine tests), A, were then used to calculate the first-order absorption rate constants, k_a, k_o and k_s, by regression analysis of the ln A values vs. time. In order to avoid or to minimize the effects of membrane absorption (Doluisio et al., 1969) or dilution (Martín-Villodre et al., 1986), the zero-time sample was not used for regression purposes.

Samples were analyzed using the diazotization and coupling procedure reported by Martín-Villodre et al., 1986.

Partition constants

Since it was observed that the partition constants gave essentially identical results as far as correlation with absorption rate constants is concerned, irrespective of whether the surfactant is present in the partition systems (Collado et al., 1988), the previously determined partition coefficients, P, between chloroform and aqueous buffer solutions of pH 7.5 or 6.2, for the tested xenobiotics (Martín-Villodre et al., 1986) were used as reference values, as was a molecular weight function of the compounds, 10^M, as will be pointed out later.

Absorption-partition correlations

Absorption and partition data were fitted to previously established equations (Martín-Villodre et al., 1986; Plá-Delfina et al., 1987; Collado et al., 1988). In summary:

(A) In the absence of surfactant (k_a, P data)

For colonic absorption:

$$k_a = \frac{k_m P^a}{B + P^a} \tag{1}$$

For small intestine absorption:

$$k_a = k_1 + k_2 = \frac{k_m P^a}{B + P^a} + \frac{k_p B'}{B' + P^{a'}} \tag{2}$$

where k_m represents the limiting asymptotic value of the membrane absorption rate constant (k_a for colon; k_1 for small intestine); k_p has the same meaning but refers to the aqueous pore absorption constant, k_2. The terms a, a', B and B' are readily calculable constants arising from the technique used (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986).

(B) In the presence of surfactant below CMC (k_o, P data)

For colonic absorption:

$$k_o = CP^d \tag{3}$$

For small intestine absorption:

$$k_o = k_{o1} + k_{o2} = CP^d + \frac{k_p B'}{B' + P^{a'}} \quad (4)$$

where k_o (colon) or k_{o1} (small intestine) is the membrane absorption rate constant; k_{o2} is equivalent to k_2 in Eqn. 2, and C and d are readily calculated constants, characteristic of the technique employed (Plá-Delfina et al., 1987; Collado et al., 1988). The symbols a' and B' have the same meaning as above.

(C) In the presence of surfactant at SMC (k_s, P data)

For colonic absorption:

$$k_s = \frac{CP^d}{1 + EP^f} \quad (5)$$

For small intestine absorption:

$$k_s = k_{s1} + k_{s2} = \frac{CP^d}{1 + EP^f} + \frac{k_p B'}{(1 + EP^f)(B' + P^{a'})} \quad (6)$$

where k_s (in colon) or k_{s1} (in small intestine) denotes the membrane absorption rate constant of the xenobiotic in the presence of surfactant at SMC; k_{s2} is the aqueous pore absorption rate constant which performs at SMC (both membrane and pore constants are usually lower than k_o or k_a, since only the free, non-micellized fraction of the compounds is available for absorption), and E and f are constants which can be calculated as previously indicated (Plá-Delfina et al., 1987; Collado et al., 1988). The remaining symbols have already been described. In Eqns. 2, 4 and 6, the coefficients B, C and E and the exponents a, d and f correspond with those in Eqns. 1, 3 and 5, respectively; however, they clearly differ in absolute value.

When correlations are established with respect to the molecular weight, M, instead of P and since a linear correlation exists between log P and M for true homologous compounds (see, for example, Plá-Delfina and Moreno, 1981), the term P in the preceding equations was substituted by 10^M, which can be considered as an 'ideal' lipophilicity index for these types of series.

The fitting operations were developed in an IBM-PC computer; the MULTI program (Yamaoka et al., 1985) was applied to fit together the interdependent equations (i.e. Eqns. 3 and 5, as well as Eqns. 2, 4 and 6). To appreciate the goodness of fits, correlation coefficients between experimental and model-predicted k_a, k_o and k_s values were calculated in all cases.

Results

The absorption rate constants determined in the presence of surfactant below and above CMC in perfusion fluids are listed in Tables 1 (tests on

TABLE 1

Absorption rate constants found in rat colon under different conditions for the tested compounds, and partition coefficients used for correlation (± S.D.)

Tested amines	DTAB concentration in the solution (w/v)			Chloroform partition coefficient [a] (pH 7.5)
	None $[k_a\ (h^{-1})]$ [a]	0.0125% (< CMC) $[k_o\ (h^{-1})]$	1% (> CMC) $[k_s\ (h^{-1})]$	
Aniline	2.766 ± 0.19	3.192 ± 0.23	2.757 ± 0.17	23.96 ± 3.15
4-Methylaniline	3.759 ± 0.44	3.470 ± 0.45	2.598 ± 0.20	83.70 ± 3.05
4-Ethylaniline	4.213 ± 0.45	3.679 ± 0.13	2.479 ± 0.20	191.08 ± 7.19
4-Propylaniline	4.356 ± 0.30	4.314 ± 0.17	2.232 ± 0.17	981.40 ± 67.9
4-Butylaniline	4.558 ± 0.37	4.880 ± 0.48	1.932 ± 0.08	2350.74 ± 99.1

[a] From Martín-Villodre et al. (1986).

TABLE 2

Absorption rate constants found in the rat small intestine in different conditions for the tested compounds, and partition coefficients used for correlation (± S.D.)

Tested amines	TTAB concentration in the solution (w/v)			Chloroform partition coefficient [a] (pH = 6.2)
	None [k_a (h^{-1})[a]]	0.0125% (CMC) [k_o (h^{-1})]	1% (CMC) [k_s (h^{-1})]	
Aniline	5.645 ± 0.49	5.495 ± 0.50	5.401 ± 0.51	26.36 ± 0.82
4-Methylaniline	6.410 ± 0.37	5.680 ± 0.56	5.457 ± 0.57	79.42 ± 1.55
4-Ethylaniline	7.375 ± 0.31	6.286 ± 0.44	5.114 ± 0.57	189.89 ± 3.61
4-Propylaniline	7.917 ± 0.22	7.443 ± 0.23	4.244 ± 0.34	752.28 ± 50.9
4-Butylaniline	6.590 ± 0.30	9.634 ± 0.42	3.552 ± 0.08	1 640.12 ± 34.4

[a] From Martín-Villodre et al. (1986).

colon) and 2 (small intestine). Both the k_o and k_s values obtained were clearly first order. Absorption constants in the absence of surfactant (k_a values) are also included in Tables 1 and 2 for the sake of comparison, as are partition coefficients, P, used for correlation (Martín-Villodre et al., 1986).

TABLE 3

Equation parameters describing the correlations between absorption rate constants found under different conditions, and partition coefficients, P

Absorption site	Variables correlated with P	Correlation equations	r	Equation parameters
Colon	k_a	1	0.996	k_m 4.534 h^{-1} a 0.938
	k_o	3	0.992	B 12.585
	k_s	5	0.972	C 2.239 d 0.0922
	k_o, k_s	3–5	0.997	E 0.0441 f 0.453
Small intestine	k_a	2	0.999	k_m 5.070 h^{-1} k_p 5.361 h^{-1} a 1.250
	k_o	4	0.999	B 780.2
	k_s	6	0.996	a' 1.838 B' 4.73×10^5
	k_a, k_o, k_s	2–4–6	0.999	C 0.00705 d 0.944 E 0.000888 f 1.021

Each set of absorption rate constants (k_a, k_o and k_s) was correlated with each of the partition constants selected (P or 10^M) by means of Eqns. 1–6. The equation parameters determined are given in Tables 3 and 4. The correlations obtained with P values have been graphically outlined in Figs. 1 (colon) and 2 (small intestine); in Fig. 3, the decomposition of the intestinal absorption rate constants (global values) into their lipophilic membrane and aqueous pore components is depicted in graphical form.

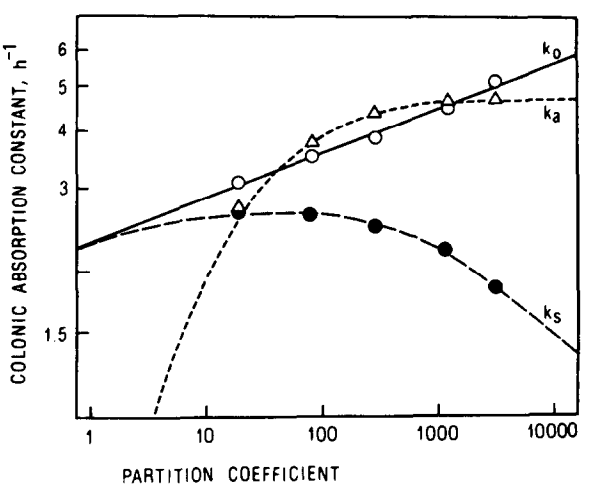

Fig. 1. Absorption-partition correlations found in rat colon, in the absence of surfactant (△), presence of DTAB below its CMC (○), and above CMC (●).

TABLE 4

Equation parameters describing the correlations between absorption rate constants found under different conditions but using a molecular weight function, 10^M, an 'ideal' partition constant directly related with partition coefficient, P

Absorption site	Variable correlated with 10^M	Correlation equation	r	Equation parameters	
Colon	k_a	1	0.998	k_m	4.536 h^{-1}
				a	0.0333
	k_o	3	0.987	B	792.58
				C	1.531
	k_s	5	0.974	d	0.00332
	k_o, k_s	3–5	0.996	E	0.00547
				f	0.0164
Small intestine				k_m	4.034 h^{-1}
	k_a	2	0.996	k_p	5.353 h^{-1}
				a	0.0438
	k_o	4	0.998	B	1.409×10^5
				a'	0.0449
	k_s	6	0.992	B'	5.386×10^6
				C	7.469×10^{-4}
	k_a, k_o, k_s	2–4–6	0.998	d	0.0266
				E	5.153×10^{-5}
				f	0.0304

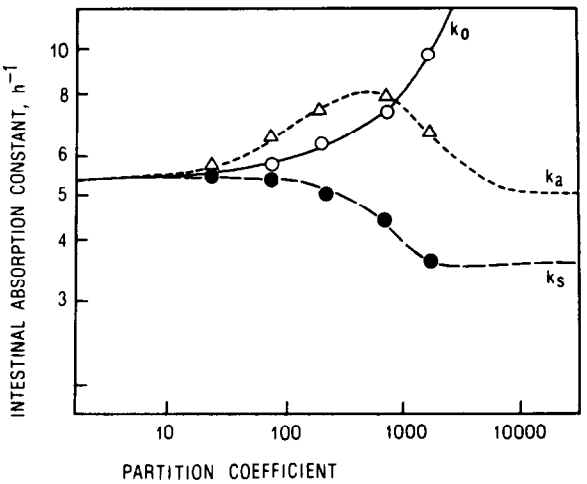

Fig. 2. Absorption-partition correlations found in rat small intestine, in the absence of surfactant, in the presence of TTAB below its CMC, and in the presence of the surfactant above CMC. The symbols are the same as in Fig. 1.

Discussion

Free solution

Absorption-partition correlations in the absence of surfactant in rat colon are hyperbolic in nature, fitting Eqn. 1, whereas in rat small in-

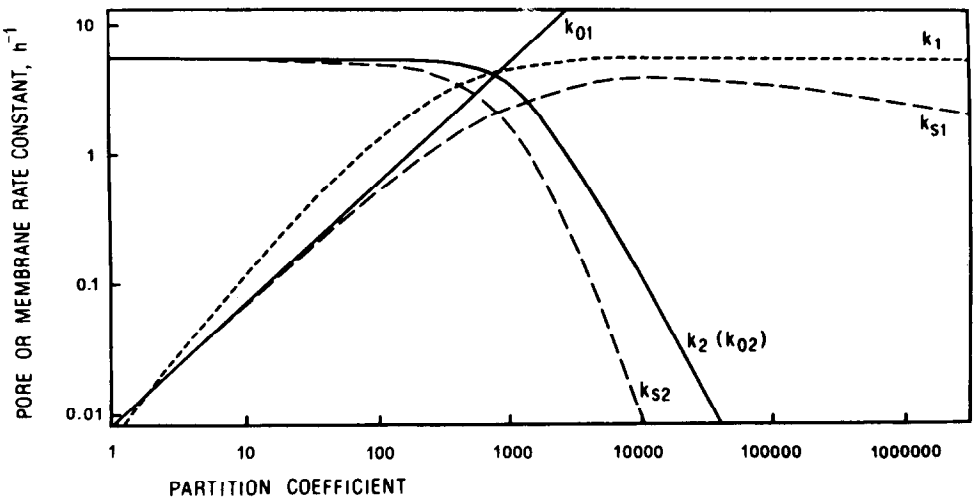

Fig. 3. Decomposition of the curves shown in Fig. 2 into their lipoidal membrane (subscript 1) and aqueous pore (subscript 2) components. Note the disposition of the membrane rate constant curves and compare them with those shown in Fig. 1.

testine the correlation is bihyperbolic and fits Eqn. 2 well (Martín-Villodre et al., 1986). This can be observed in Figs. 1 and 2, respectively (k_a lines). For intestinal absorption, two rate constants which act simultaneously are considered, i.e. k_1, which accounts for the lipophilic membrane penetration pathway, and k_2, for the aqueous pore diffusion route. The sum of these two rate constants gives the value of the global absorption constant, k_a. With increasing partition coefficient, or molecular weight, k_1 also increases until its asymptotic value, k_m, is attained, whereas k_2 exhibits a decrease from its asymptotic value, k_p, to zero because the solute molecules or ions are progressively impeded for pore penetration.

Neither type of correlation is observed when polysorbate 80 is present in perfusion solutions at or below CMC or at SMC, giving rise to Eqns. 3 and 4 or 5 and 6, respectively (Plá-Delfina et al., 1987; Collado et al., 1988). This is exactly what occurs when cationic surfactants are used instead of nonionic, as will be shown immediately.

Cationics below CMC

Equations established for *colon absorption* data can be readily interpreted in light of previous reported procedures (Plá-Delfina et al., 1987); the results are graphically represented in Fig. 1 (k_o line). The presence of DTAB below CMC gives rise to a linear and double-logarithmic correlation between k_o and P, which can be explained if we assume that the surfactant removes the limiting effect of the aqueous diffusion layer adjacent to the membrane (i.e. k_m cancels out), and increases membrane polarity (an effect which tends to decrease the expected slope of the line).

Nevertheless, it should be pointed out that with the cationic surfactant, the k_o, P correlation line intersects the hyperbola found in the absence of surfactant which correlates k_a and P values. This means that some absorption rate constants, k_o (for compounds of intermediate lipophilicity) are somewhat lower than their corresponding k_a, a phenomenon which was not observed with the nonionic polysorbate. This particular behavior could be attributed to a much more drastic change in polarity of the absorbent membrane, so that such compounds are less permeable than with polysorbate due to their intrinsic lipophilicity. As the apparent lipophilicity increases and because of the absence of a limiting factor for diffusion for these less polar compounds, the k_o values undergo a slow and progressive increase.

In the case of the rat, *small intestine absorption*, if it is assumed that the surfactant does not substantially interfere with pore penetration, i.e. that $k_{o2} \approx k_2$ (Collado et al., 1988), the membrane absorption rate constants, k_{o1} (equivalent, obviously, to $k_o - k_{o2}$), can be calculated as the first addends of Eqn. 4; k_{o1} values give a linear and double-logarithmic correlation with P or 10^M as k_o does for colon tests (Fig. 3, k_{o1} line), whereas k_{o2}, P correlation remains inversely hyperbolic (k_{o2} line in Fig. 3). Correlations between global intestinal absorption rate constants, k_o, and partition constants are thus very complex in appearance (Fig. 2) but are readily explained in light of the preceding reasoning. This has been believed to confirm the two main effects of the surfactants on the absorbing membrane and its environment when perfused at or below its CMC, as discussed. Again, as in colon tests, the k_{o1}, P correlation line intersects the membrane hyperbola found in the absence of surfactant (Fig. 3, k_1 line); this can be also explained as resulting from more extensive alteration in membrane permeability by cationics as compared to polysorbate.

Cationics above CMC

The previously reported interpretation of the correlations found between absorption rate constants and lipophilicity parameters in the presence of the nonionic polysorbate at its SMC (Plá-Delfina et al., 1987; Collado et al., 1988) can be fully applied to the reported data. Briefly, the presence of micelles leads to equilibrium of the xenobiotic between the internal lipophilic micellar phase and free aqueous solution, which is governed by general partitioning laws. Thus an 'internal' partition coefficient, P_a, leading to the appearance of micelle-solubilized and free amine fractions (F_m and F_f, respectively) should be considered. Since the latter fraction is absorbed, a multiple global equilibrium appears in the whole system, which becomes heterogeneous and gives rise to bilinear correlations between membrane absorption rate

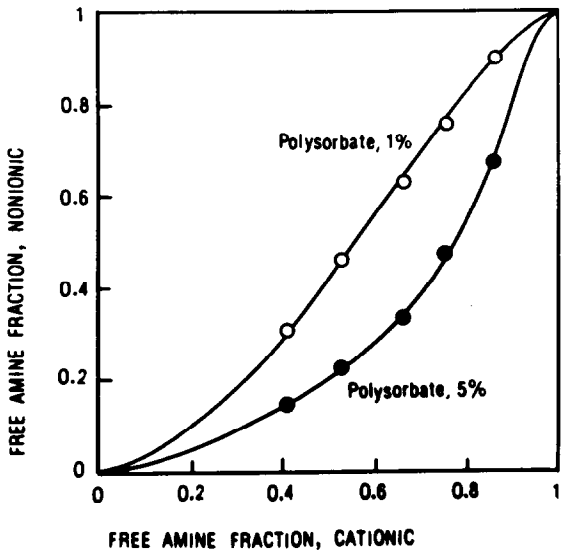

Fig. 4. Correlation between free (nonmicellized) amine fractions, F_f, found in the presence of the cationic surfactant DTAB, at 1% in colonic perfusion fluid, and in the presence of polysorbate 80 at 1 and 5% in the solution (latter, reported by Plá-Delfina et al., 1987).

constants (k_s in colon; k_{s1} in small intestine) and lipophilicity parameters. Pore absorption rate constants in the small intestine, k_{s2}, are reduced when compared with those determined in the presence of surfactant at CMC or in free solution, since only F_f is available to both absorption routes (i.e. $k_{s2} = k_{o2} F_f$).

The internal partition coefficients estimated in the presence of cationics at SMC, as well as the free and solubilized amine fractions, are consistent with this approach in both absorption sites. The P_a values ascertained at the working surfactant concentrations are lower than those observed with polysorbate at 5% concentration, and, consequently, free amine fractions remaining in the luminal fluid are greater. On the other hand, the correlation coefficients (cationic vs. nonionic) found for P_a, F_f and F_m, agree quite well ($r = 0.998$ in colon; $r = 0.984$ in small intestine). In Fig. 4 some of the correlations obtained for F_f values are depicted graphically.

As expected, k_s colonic constants are correlated with P or 10^M through a bilinear equation curve, as shown in Fig. 1 (k_s line), whereas for small intestine a similar correlation is obtained for the membrane absorption constants, k_{s1} (i.e. $k_{o1} F_f$), as shown in Fig. 3 (k_{s1} line). In both cases, the left arms of the bilinear curves tend to run together with the k_o or k_{o1} lines, i.e. when the increasing hydrophilicity of the compounds prevents their micelle solubilization. Aqueous pore rate constants, k_{s2}, are, obviously, lower than those found below CMC or in free solution, as pointed out above (see k_{s2} line in Fig. 3). In view of these reasons, the apparently complex correlation found in the small intestine between global k_s values and partition constants, shown in Fig. 2, can be easily understood.

Comparative behaviour of cationics vs. nonionics

In conclusion, the effects of the cationic surfactants tested here on xenobiotic absorption are parallel to those found for polysorbate, so it may be that the mechanisms involved in absorption are the same. Only a minor difference has been observed: the change in membrane polarity is, apparently, more effective with cationics for this particular series of xenobiotics, leading to k_o values that are somewhat lower than k_a for compounds of intermediate lipophilicity, in both small intestine and colon. If similar behaviour were to be demonstrated for other xenobiotic series, anionic or nonionic in nature, and for other surfactant types, it could be reasonably inferred that the mechanisms postulated here are general and can be used for predictive purposes in order to prevent interactions and, perhaps, to improve the bioavailability of some drugs.

Acknowledgments

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