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Compared effects of synthetic and natural bile acid surfactants on xenobiotic absorption: IV. Studies with taurocholate in the rat small intestine

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Abstract

Fundamental differences in behavior between synthetic surfactants and taurocholate as a natural model surfactant, relative to their influences on xenobiotic absorption, previously found in rat colon, are now also shown to exist in the small intestine. These differences have been revealed using the absorption-lipophilicity correlation approach, with a homologous phenylalkylcarboxylic acid series. In order to prevent extensive intrinsic absorption of taurocholate, which could mask its true mechanisms of actuation, the proximal half of the rat small intestine was used for absorption tests, after examination of the absorption potentialities of the different small intestinal fractions. The biophysical model describing xenobiotic absorption in the presence of taurocholate at its critical micelle concentration (CMC) does not change relative to that found for xenobiotics in free solution, which are clearly bihyperbolic in nature. At supramicellar concentration (SMC) and in the presence of mixed micelles with lecithin, taurocholate leads to a bilinear correlation for membrane permeation. This means that the effect of the natural surfactant at the CMC on xenobiotic absorption is unimportant, while at the SMC a micelle-solubilizing effect, which is reinforced in the presence of mixed micelles, is evident. On this basis, a general assumption is made to explain the in vivo absorption promoting capacity of bile salts for some lipophilic drugs.

Key words: Bile salt; Surfactant; Taurocholate; Intestinal absorption; Micelle solubilization; Biophysical model

1. Introduction

In previous papers (Bermejo et al., 1991; Garrigues et al., 1994) the effects of natural bile acid surfactants on the absorption of acid xenobiotics in rat colon were studied and compared with those of synthetic surfactants in the same absorption site. The homologous series of compounds formed by phenylalkylcarboxylates and the absorption-lipophilicity approach (Plá-Delfina et al., 1987) were used for this purpose. The results showed that synthetic and natural surfactants behave as quite different biopharmaceutical species, as indicated by the following observations:

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(a) Whereas synthetics, at their critical micelle concentration (CMC) or even below it, were shown to disrupt the aqueous stagnant diffusion layer adjacent to the membrane, bile acid surfactants did not. Rather, they seemed to slightly reinforce its limiting effect on xenobiotic absorption.

(b) Bile acid surfactants can increase membrane polarity, thus leading to an increase in absorption for highly hydrophilic compounds, however, this effect appears as much less marked than that exerted by synthetics.

(c) At supramicellar concentrations (SMC), the micelle-solubilizing effect of natural bile acids was shown to be much weaker for xenobiotics than that found for synthetics, although this effect was considerably reinforced by the presence of mixed micelles with lecithin.

Accordingly, the global effects on the absorption of drugs and xenobiotics in general are expected to be quite different for each type of surfactants, although both kinds can be useful as absorption promoters under suitable conditions.

In the present paper, the effects of the natural bile salt surfactant sodium taurocholate on the absorption of the previously tested series of xenobiotics, phenylalkylcarboxylates, in the small intestine of the classical in situ rat gut preparation have been studied. Our purpose was to extend our previous knowledge by examining the behavior of this highly specialized absorption site, where the absorption-lipophilicity correlations were expected to be much more complex than in the colon because of the coexistence of two simultaneous lipoidal membrane and aqueous pore absorption pathways (Plá-Delfina and Moreno-Dalmau, 1981). Moreover, the presumed absorption of the natural surfactant itself, which is not as negligible as was found in the colon experiments (Bermejo et al., 1991), can also make the interpretation of the results very difficult. Bile acids are, indeed, effectively absorbed in the intestine by means of both passive and active processes, as has been shown, among many others, by Dietschy (1968) and Wilson (1981). These progressive losses in the luminal concentration of surfactant should be reflected in parallel losses in action of both its free and micellar forms on xenobiotic absorption. Therefore, care should be taken to select the intestinal fraction in which the mechanisms of action of bile salts on xenobiotic absorption become most evident, i.e., the one showing a minimal absorption rate for the surfactant; otherwise, the data generated can lead to misinterpretations about these general mechanisms.

2. Materials and methods

2.1. Xenobiotics and surfactant

Seven ω-phenylalkylcarboxylic acids used in previous colon experiments (Bermejo et al., 1991; Garrigues et al., 1994), from phenylacetic to phenylcaprylic, were used as model xenobiotics. As a natural surfactant, sodium taurocholate was selected (purchased from Sigma Chemical Co., with a purity of 98.0%). The CMC of the surfactant under the test conditions was determined previously by plotting surface tension (as measured by means of the ring method in a Lauda Model 7201 tensiometer, at 20°C) against surfactant concentration in a pH 6.4 perfusion fluid, and was shown to be 4.8 mM.

2.2. General absorption technique

The in situ rat gut preparation (Doluisio et al., 1969; Martín-Villodre et al., 1986), adapted to the use of selected intestinal segments (Merino et al., 1989; Sánchez-Picó et al., 1989) was performed with male Wistar rats weighing from 200 to 300 g after fasting for 20 h. Test solutions (5–7 ml) were perfused in the selected fraction, and the concentration of solute remaining in the luminal solution was determined through 0.1 or 0.2 ml samples taken every 5 min for a total time of 25 or 30 min, depending on the test series. Solutions were prepared by dissolving a fixed amount of the xenobiotic and/or the surfactant under study in isotonic saline buffered to the suitable pH by adding 1% (v/v) Sörensen phosphate made isotonic prior to use. Immediately before perfusion,

the pH of the solution was adjusted exactly, and tonicity assessed with the aid of a Halb-Micro Haver osmometer. In proximal segment tests, the bile duct was ligated in order to prevent enterohepatic cycles. A moderate but significant reduction in the volume of the perfused solutions was observed at the end of the sampling periods. Water reabsorption was therefore corrected according to a previously reported procedure based on the direct measurement, at fixed times, of the remaining volumes of the test solutions perfused independently in selected animals (Martín-Villodre et al., 1986; Sánchez-Picó et al., 1989). The corrected remaining concentrations, A, were then used for the calculation of the apparent first-order absorption rate constants by regression analysis of the natural logarithms of A vs time. In order to minimize membrane adsorption and sample dilution effects (Doluisio et al., 1970; Martín-Villodre et al., 1986) the zero-time nonperfused samples were not taken into account for regression.

2.3. Intrinsic taurocholate absorption tests

As indicated above, the proper selection of the intestinal fraction in which the absorption of taurocholate was lowest was desirable. For this purpose, two separate series of experiments were carried out.

2.3.1. Phase I experiments

The entire length of the rat small intestine (about 1 m) was divided in three segments of equal lengths, measuring about 33 cm, which were designated as proximal, middle and distal thirds, and perfusion was performed in one of these segments. The pH of the perfusion fluid had been previously adjusted to the average value found for each segment in three preliminary rat tests performed independently by perfusion for 30 min of a non-buffered blank solution in each intestinal segment, as shown in Table 1. 5 ml of 4.8 mM taurocholate solutions buffered to the corresponding pH were then perfused in each intestinal fraction for a total time of 30 min, through 0.1 ml samples, taken every 5 min, and the absorption rate constants were calculated and corrected for water reabsorption.

2.3.2. Phase II experiments

Since the phase I results demonstrated the convenience of using a proximal intestinal fraction, and in order to facilitate experimental work with xenobiotic solutions, the first proximal half of the rat small intestine (i.e., the first 50 cm segment) was tested for taurocholate absorption through a series of five rat experiments. 7 ml of a 4.8 mM taurocholate solution, buffered to pH 6.4 (as determined prior to absorption tests, as above) were perfused in this intestinal segment for a total time of 30 min, with 0.2 ml samples taken each 5 min. Absorption rate constants were then calculated, corrected for reabsorption and averaged. Finally, this half-proximal intestinal segment was selected for absorption tests with xenobiotics.

Table 1
Intrinsic corrected absorption rate constants for taurocholate in the selected intestinal segments, and pH values found for each fraction

Intestinal segment and length	Number of animals	pH of perfusion fluid ($\pm\sigma$)	Absorption constant of taurocholate ($\pm\sigma$) (h^{-1})
Proximal third (33 cm)	3	6.2 (0.1)	0.357 (0.028)
Middle third (33 cm)	3	6.9 (0.1)	0.997 (0.201)
Distal third (33 cm)	3	7.8 (0.1)	4.157 (0.564) [a]
Proximal half (50 cm)	5	6.4 (0.1)	0.531 (0.091)

[a] Significantly different from proximal and middle ($p < 0.05$; Scheffé test).

2.4. Absorption of xenobiotics under different conditions

Perfusion concentrations ranging from 0.05 to 1.0 mg/ml were employed for acids, depending on their solubility. Four series of experiments were carried out in the half-proximal segment of the rat small intestine: (1) in the absence of surfactant, i.e., in free solution (k_a values); (2) in the presence of taurocholate 4.8 mM (k_o values); (3) in the presence of taurocholate at the SMC, which was 9.0 mM (k_s values), and (4) in the presence of mixed micelles of taurocholate 9.0 mM and lecithin 3.3 mM (these latter was purchased from Sigma Chemical Co. with 98.0% purity) (k_{sm} values). The perfused volume was 7 ml (pH 6.4) and 0.2 ml samples were taken every 5 min, for a total time of 25 min.

2.5. Lipophilicity constants

HPLC capacity factors, K', found for the acids at pH 6.2 (Fabra-Campos et al., 1991) were used as lipophilicity constants. Moreover, the number of carbon atoms in the side chain, N, was used, since it represents an error-free lipophilicity index, linearly related to log P for true homologous series of xenobiotics. Obviously, the value 10^N should be used instead of the partition coefficient, P, in these cases.

2.6. Analytical technique

2.6.1. Taurocholate determinations

An HPLC method was used to quantify the natural surfactant in samples corresponding to intrinsic absorption tests, with the following components: a Waters Model 540 pump, a Waters Model U6K injector, the Lambda-Max Model 481 detector, set at 205 nm, and a Perkin-Elmer LCI-101 integrator, with a Spherisorb C-18 column (150 × 4.6 mm) of 5 μm particle size. The mobile phase was a mixture of methanol/acetonitrile/aqueous phosphate buffer (pH 2.5) in v/v proportions of 65:20:70, at a flux of 1 ml/min. The retention time for taurocholate was found to be 3.4 min. Calibration lines were excellent ($r >$ 0.999), with intercepts not significantly different from zero and coefficients of variation ranging from 0.2 to 0.9.

Table 2
Volumetric composition of the mobile phases for HPLC determination of xenobiotics and retention times, t_r, found for each compound

Tested acids	Acetonitrile:pH 3.0 acetate buffer	Retention time (min)
Phenylacetic	40:60	3.2
Phenylpropionic	30:70 [a]	3.8
Phenylbutyric	50:50	3.5
Phenylvaleric	45:55	6.0
Phenylcaproic	55:45	5.6
Phenyloenanthic	55:45	6.2
Phenylcaprylic	55:45	8.0

[a] pH 5.0 acetate buffer.

2.6.2. Xenobiotic determinations

The same chromatographic components and conditions as for taurocholate determinations were used for the acidic xenobiotics, except that the detector was set at 258 nm and the mobile phases were mixtures of aqueous acetate buffer (pH 3.0) and acetonitrile in the volumetric proportions shown in Table 2, where the retention times found for each acid are also listed. Calibration lines for each compound and condition were obtained, without significant intercept and with correlation coefficients of more than 0.999. Coefficients of variation ranging from 0.2 to 7.9 were obtained.

2.7. Fitting equations to data

Absorption/lipophilicity data found in the absence of surfactant were correlated through the general bihyperbolic equation, as reported previously (Collado et al., 1988; Fabra-Campos et al., 1991):

$$k_a = k_1 + k_2 = \frac{k_m \cdot P^a}{B + P^a} + \frac{k_p \cdot B'}{B' + P^{a'}} \quad (1)$$

where k_m and k_p are the limiting asymptotic values for the rate constants governing lipoidal membrane (k_1) and aqueous pore (k_2) absorp-

tion, P represents the lipophilicity constant used (K' or 10^N), and the terms a, B, a' and B' are constants of the technique, readily estimated through logit/log regression of k_1 or k_2 against P, with further computer optimization (Plá-Delfina and Moreno-Dalmau, 1981).

For the data obtained in the presence of taurocholate at CMC, the following equation was operative:

$$k_o = k_{o1} + k_{o2} = \frac{k'_m \cdot P^c}{D + P^c} + \frac{k_p \cdot B'}{B' + P^{a'}} \quad (2)$$

where the symbols have the same meaning as above, since the biophysical model does not change, as justified later. Symbols for pore diffusion do not change relative to free solution, whereas for membrane permeation, k'_m, c and D are the counterparts of k_m, a and B, respectively, in Eq. 1.

For the data found in the presence of taurocholate at SMC, and in the presence of mixed micelles, the following equations were operative:

$$k_s = k_{s1} + k_{s2} = \frac{k'_m \cdot P^c}{(1 + E \cdot P^f)(D + P^c)} + \frac{k_p \cdot B'}{(1 + E \cdot P^f)(B' + P^{a'})} \quad (3)$$

and:

$$k_{sm} = k_{sm1} + k_{sm2} = \frac{k'_m \cdot P^c}{(1 + E' \cdot P^{f'})(D + P^c)} + \frac{k_p \cdot B'}{(1 + E' \cdot P^{f'})(B' + P^{a'})} \quad (4)$$

where the symbols have the same meaning as above excepting E, f, E' and f', which are constants related to internal partition coefficients (micelle/solution), and dependent on the technique, as pointed out later.

Fitting was carried out with the aid of the PCNONLIN (V3.0) program. A simultaneous fit was developed to estimate common parameters. To evaluate the goodness of the fit, the squared sums of residuals (SSQ) and correlation coefficients between experimental and model-predicted values (r) were calculated.

3. Results

In Table 1 the absorption rate constants found for taurocholate in the third and half segments of the rat small intestine, corrected for water reabsorption, as well as the working pH values in each

Table 3
Absorption rate constants of xenobiotics found in different perfusion conditions in the proximal half of the rat small intestine, already corrected for water reabsorption, and partition constants used for correlation through Eq. 1–4

Tested acid	Absorption rate constant (h^{-1})				Partition constant (K') [a]
	Free solution (k_a)	CMC (4.8 mM) (k_o)	SMC (9.0 mM) (k_s)	Mixed micelles (k_{sm})	
Phenylacetic	5.677 (0.280)	5.958 (0.493)	5.652 (0.469)	6.404 (0.356)	0.248 (0.008)
Phenylpropionic	6.864 (0.364)	6.897 (0.776)	6.781 (0.536)	6.745 (0.429)	0.498 (0.016)
Phenylbutyric	6.926 (0.266)	6.723 (0.191)	6.951 (0.293)	7.044 (0.347)	0.980 (0.027)
Phenylvaleric	6.538 (0.452)	6.682 (0.522)	6.681 (0.626)	6.407 (0.301)	1.936 (0.056)
Phenylcaproic	6.528 (0.652)	6.614 (0.739)	6.471 (0.244)	6.163 (0.579)	3.819 (0.028)
Phenyloenanthic	6.579 (0.614)	6.585 (0.399)	6.330 (0.316)	5.365 (0.384)	8.015 (0.072)
Phenylcaprylic	6.537 (0.563)	6.512 (0.378)	5.943 (0.389)	4.291 (0.229)	16.570 (0.124)

[a] From Fabra-Campos et al. (1991).
Values within parentheses represent the standard deviations. Constants are average values for six animals.

Table 4
Equation parameters found for absorption-lipophilicity correlations

Equation parameters	Correlation between K' and:				Correlation between 10^N and:			
	k_a	k_o	k_s	k_{sm}	k_a	k_o	k_s	k_{sm}
k_m (h^{-1})	6.4657	–	–	–	6.5136	–	–	–
k'_m (h^{-1})	–	6.5299	6.5299	6.5299	–	6.5717	6.5717	6.5717
k_p (h^{-1})	1.1740	1.1740	1.1740	1.1740	0.8220	0.8220	0.8200	0.8220
a	3.4983	–	–	–	0.6942	–	–	–
a'	1.3378	1.3378	1.3378	1.3378	0.8787	0.8787	0.8787	0.8787
B	0.0027	–	–	–	8.2762	–	–	–
B'	0.4998	0.4998	0.4998	0.4998	5.0×10^3	5.0×10^3	5.0×10^3	5.0×10^3
c	–	2.3899	2.3899	2.3899	–	0.5205	0.5205	0.5205
D	–	0.0099	0.0099	0.0099	–	2.9209	2.9209	2.9209
E	–	–	0.0033	–	–	–	3.0×10^{-5}	–
E'	–	–	–	0.0136	–	–	–	2.2×10^{-4}
f	–	–	1.2107	–	–	–	0.4441	–
f'	–	–	–	1.3084	–	–	–	0.4250
	SSQ = 0.4733 r = 0.974				SSQ = 0.4255 r = 0.977			

Symbols are the same as those of Eq. 1–4. Statistical figures are also shown (global fit).

segment, are shown. As pointed out above, the proximal half was selected for tests with xenobiotics under all conditions. The absorption rate constants determined in the different series of experiments for every acid tested are listed in Table 3, where the previously determined HPLC

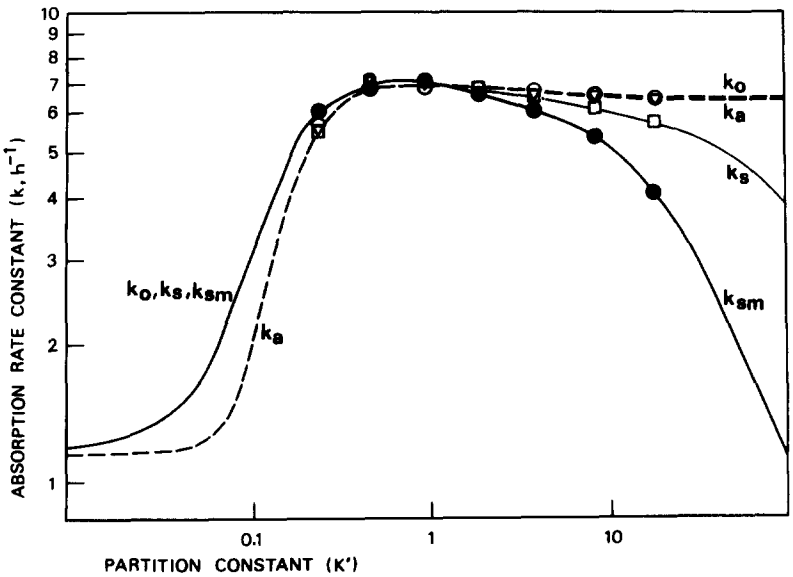

Fig. 1. Plots of the global absorption rate constants, k, found for xenobiotics in the proximal half of the rat small intestine vs lipophilicity constants, K'. The k values were obtained in free solution (k_a (▽), Eq. 1), in the presence of the natural surfactant at the CMC (k_o (○), Eq. 2), in the presence of taurocholate at the SMC (k_s (□), Eq. 3), and also in the presence of mixed micelles of taurocholate/lecithin (k_{sm} (●), Eq. 4).

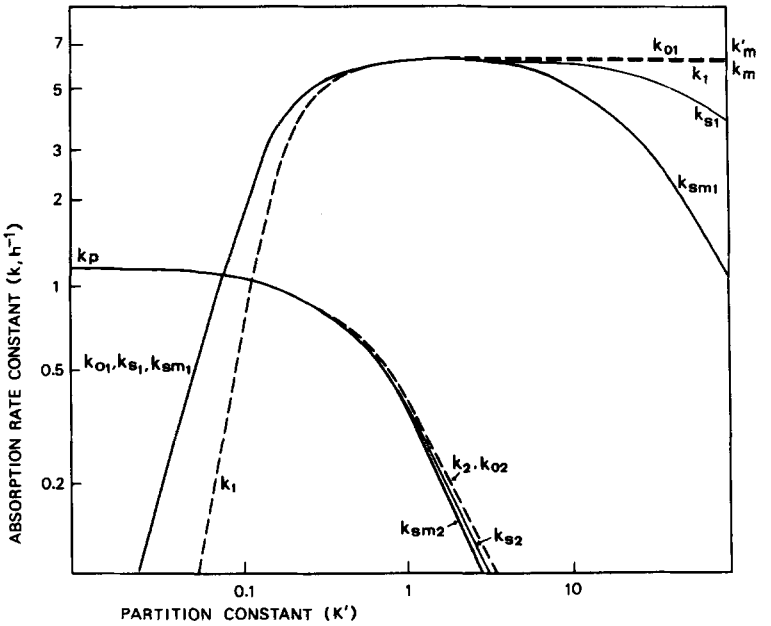

Fig. 2. Decomposition of the curves shown in Fig. 1 into their lipoidal membrane (subindexes 1) and aqueous pore (subindexes 2) absorption components.

partition constants, K', are also shown. Absorption-lipophilicity correlation fits gave equation parameters and statistical figures shown in Table 4. In Fig. 1, the global correlations are graphically outlined, on the basis of K' values as partition constants. Decomposition of the absorption rate constants into the lipoidal membrane and aqueous pore components, as the correlations are concerned, is shown in Fig. 2.

4. Discussion

4.1. Intrinsic taurocholate absorption

From the data of Table 1, it becomes evident that the intrinsic absorption of taurocholate is much greater in the distal segment of the rat small intestine, undoubtedly due to the presence of bile acid carriers along this segment, as pointed out elsewhere. Although the smaller absorption rate constant was found in the proximal third of small intestine, for practical purposes (i.e., to facilitate biological work and analytical determinations) it would be desirable to use a 50 cm fraction of intestine. On the other hand, it could be assumed that the reduction of the luminal taurocholate concentration by absorption in the proximal half of the rat small intestine (where $k_a = 0.0088$ min^{-1}, which represents a reduction of less than 20% at 25 min) would not presumably have a significant effect on xenobiotic absorption results in the presence of taurocholate, as far as the mechanisms of actuation of the surfactant are concerned. Therefore, the half-proximal segment of the rat small intestine was selected for absorption tests with xenobiotics.

4.2. Xenobiotic absorption in free solution

The results obtained here for xenobiotic absorption in the absence of surfactant in the perfusion fluid fully confirm the bihyperbolic model predictions, as stated by Plá-Delfina and Moreno-Dalmau (1981). Eq. 1 fits these data very well (i.e., k_a values against K' or 10^N, as shown in Table 4). As occurred with the colon tests (Bermejo et al., 1991) as well as in the whole rat small intestine (Fabra-Campos et al., 1991), the k_a values found for xenobiotics are much higher

than expected if one takes into account the rather high degree of ionization of the acids (pK_a ranging from 4.3 to 4.8) at the working pH. This behavior can be attributed mainly to membrane permeation ($k_m = 6.5\ h^{-1}$) and indicates some absorption of the ionic species, as occurs with other organic substances (Plá-Delfina et al., 1980; Casabó et al., 1987; Miralles-Loyola et al., 1991). It cannot be due to aqueous pore penetration, since the pore absorption pathway, as compared with other compound series, is hardly operative (k_p ranging from 0.8 to 1.2 h^{-1}); the same was found to be true in the whole small intestine (Fabra-Campos et al., 1991). Again, the degree of ionization of the acids, as compared with other compounds tested such as substituted anilines (Martín-Villodre et al., 1986), is presumably responsible for that phenomenon via attraction-repulsion interactions between the ionized species and the pore charges (Casabó et al., 1987; Fabra-Campos et al., 1991; Miralles-Loyola et al., 1991). In conclusion, the results reported here would confirm the behavior observed for other non-ionic and basic series of xenobiotics (Plá-Delfina and Moreno-Dalmau, 1981; Martín-Villodre et al., 1986; Casabó et al., 1987; Miralles-Loyola 1991), and, in fact, constitute a further validation of the functional bihyperbolic expression (Eq. 1) as a general biophysical absorption model in the small intestine.

4.3. Xenobiotic absorption in the presence of surfactant at CMC

The absorption data obtained under these conditions (Table 3, k_o values) vs partition constants were not satisfactorily fitted by the previously established equation for synthetic surfactants (Collado et al., 1988; Fabra-Campos et al., 1991; Martínez-Coscollá et al., 1993):

$$k_o = k_{o1} + k_{o2} = C \cdot P^d + \frac{k_p \cdot B'}{B' + P^{a'}} \qquad (5)$$

Since in colon tests it was shown that for membrane absorption in the presence of taurocholate at its CMC the first term, in Eq. 1, was operative, i.e., k_{o1} behaved as k_1 in terms of membrane diffusion (Bermejo et al., 1991), and since Collado et al. (1988) and Fabra-Campos et al. (1991) were not able to find any influence of surfactant on the aqueous pore absorption pathway (i.e., $k_{o2} \approx k_2$), it was assumed that to describe the global xenobiotic absorption behavior in the presence of taurocholate at its CMC, Eq. 2 would have to be applied. This was fully confirmed through computer-fitting operations, and would mean that the surfactant at the CMC is not able to disrupt the aqueous diffusion layer adjacent to the luminal side of the intestinal absorbent membrane. Moreover, as the asymptotic value for membrane absorption, k'_m, was found to be of the same order of magnitude as that found for xenobiotics in free solution, k_m (see Table 4), it was concluded that, at the CMC, taurocholate did not substantially increase membrane polarity in this particular instance. This contrasts with what occurred in colon tests, where taurocholate seemed to slightly reinforce the resistance of the aqueous diffusion layer to solute permeation, probably via an increase in membrane polarity (Bermejo et al., 1991; Garrigues et al., 1994). In the small intestine, the increase in membrane polarity appears to be much less efficient and would affect only the highly hydrophilic compounds. This can be seen in Fig. 2, where the left branch of the curve representing the membrane absorption pathway of xenobiotics in the presence of taurocholate at the CMC (k_{o1} line) runs somewhat over that found for the compounds in free solution (k_1 line). As far as the tested compounds are concerned, however, this effect on polarity can be seen as unimportant and only slightly detectable for phenylacetic acid, for which, notwithstanding, k_o was not significantly different from k_a ($p > 0.05$ through the Scheffé test); for the remaining elements (middle and right branches of the k_{o1} curve) there are no significant differences between k_o and k_a values either, as can be expected. Be that as it may, these results are consistent with the generalized opinion according to which the aqueous boundary layer properties are more affected by bile acid surfactants in colon and rectum than in small intestine (Poelma et al., 1989). This contrasts with the much more conspicuous effects that have been observed for synthetic surfactants in the

small intestine, characterized by a significant increase in absorption for hydrophilic and lipophilic compounds, which were attributed to an increase in membrane polarity and to a disruption of the aqueous boundary layer, respectively (Collado et al., 1988; Fabra-Campos et al., 1991).

4.4. Xenobiotic absorption with surfactant at the SMC and mixed micelles

Both membrane and pore components leading to xenobiotic absorption should be influenced by micellar solubilization. As shown by Collado et al. (1988), a correction factor for k_s values, relative to k_o, should be applied: $1/(1+P_i)$, where P_i represents the internal partition coefficient of the xenobiotics between surfactant micelles and free solution, easily calculable from experimental data. Since the internal partition coefficient can be related to the partition constant selected, P, by means of the Collander equation (i.e., $P_i = E \cdot P^f$ for taurocholate at the SMC, and $P_i = E' \cdot Pf'$ for mixed micelles), Eq. 3 and 4, respectively, should be operative to describe xenobiotic absorption in these cases. This was fully confirmed through computer-fitting operations (Table 4). As shown in Fig. 2, the pore penetration pathway of xenobiotics in the presence of taurocholate at the SMC, as well as in the form of mixed micelles (k_{s2} and k_{sm2}, respectively) is virtually not modified relative to that found in free solution (k_2) or in the presence of the surfactant at the CMC (k_{o2}). This is undoubtedly indicative of the very weak or even negligible micelle-solubilizing capacity of taurocholate for hydrophilic and weakly lipophilic elements of the tested series, the most available for pore penetration as well. As far as the membrane permeation pathway is concerned, it can be observed from Fig. 2 that the left branches of the k_{s1} and k_{sm1} lines coincide with that obtained at the CMC (k_{o1} line), hence, it can be concluded that the slight increase in membrane polarity provided by the surfactant is maintained. The picture changes for the right branches of the k_{s1} and k_{sm1} lines, which tend to descend relative to those obtained for k_1 and k_{o1}, particularly in the case of mixed micelles (k_{sm1}). This is no more than a consequence of the increasing micellar solubilization of the compounds as their lipophilicity increases, leading to a bilinear membrane absorption-lipophilicity correlation, and thus making apparent the heterogeneous character of the system. As compared to synthetic surfactants, the micelle-solubilizing ability of the natural surfactant is clearly biased for highly lipophilic compounds of the series, i.e., the differences in k_{sm} values relative to those of k_a/k_o become significant only for phenyloenantic and for phenylcaprylic acids ($p < 0.05$, through the Scheffé test). In conclusion, the absorption model in the presence of a micellar phase undoubtedly changes from homogeneous to heterogeneous as a consequence of the solubilizing capacity of taurocholate, particularly in the form of mixed micelles with lecithin. This is demonstrated by the fact that the bilinear equation is the best for fitting k_{s1} and k_{sm1} values against partition constants, as can be seen in Table 4. In the opinion of the authors, this is the most relevant feature of taurocholate as an absorption modifier.

4.5. In vivo biopharmaceutical implications

In view of the effects observed for taurocholate on the intestinal absorption of the tested xenobiotics, which are practically lacking at the CMC and characterized by a rather moderate micelle-solubilizing capacity at the SMC, and taking into account that, in practice, enhanced absorption not only for fat and cholesterol (Simmonds, 1972; Feldman et al., 1975; Watt and Simmonds, 1984) but also for some lipophilic drugs (Muranishi, 1985; Del Estal et al., 1991) has been conclusively demonstrated and attributed to in vivo solubilization by bile salts, our conclusion, as advanced in a recent communication (Segura-Bono et al., 1994) is that, apart from the well-known wetting and emulsifying actions of the bile salts on hydrophobic drug particles and the natural fats, whenever the compounds are sufficiently lipophilic to be trapped into the micellar phase, the increased absorption observed can be due to: (1) a previous in vivo solubilization of the compound in the bile salt mixed micelles; and (2) a subsequent breaking of the micelles due to a progressive disappearance of the bile salt

molecules, via absorption, mainly in the distal fraction of the small intestine, since the micellar bile salt is in equilibrium with the free one in the aqueous membrane environment. This would lead to an effective release, in situ, of the solubilized compound in a molecularly micronized, readily absorbable form. This would be, of course, a merely functional explanation of the phenomenon, independent of the mechanism of bile acid absorption and their conditioning factors, such as the mobility across the aqueous diffusion layer or the pH of the membrane environment (Shiau, 1981).

In contrast, for the synthetic surfactants, the micelle-solubilizing effect on the compounds would remain quite stable in all parts of the intestine, since no systemic absorption of the surfactant is produced along its entire length. When mixtures of natural and synthetic surfactants are used, the effect of bile salts could be negated since the synthetic surfactant micelles will retain their drug-solubilizing capacity, even when natural surfactant is absorbed.

Comparative in vivo oral bioavailability tests on micelle-solubilized lipophilic drugs by synthetic and natural surfactants are being carried out in our laboratory division in order to test this assumption, as well as in situ absorption studies in rat ileum as confirmative complementary tests.

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