Adsorption Properties of Polar/Apolar Inducers at a Charged Interface and their Relevance to Leukemia Cell Differentiation

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ABSTRACT The interfacial adsorption properties of polar/apolar inducers of cell differentiation (PAIs) were studied on a mercury electrode. This study, on a clean and reproducible charged surface, unraveled the purely physical interactions among these compounds and the surface, apart from the complexity of the biological membrane. The interfacial behavior of two classical inducers, hexamethylenebisacetamide (HMBA) and dimethylsulfoxide, was compared with that of a typical apolar aliphatic compound, 1-octanol, that has a similar hydrophobic moiety as HMBA but a much smaller dipolar moment. Both HMBA and Octanol adsorb flat in contact with the surface because of hydrophobic forces, with a very similar free energy of adsorption. However, the ratio of polar to apolar moieties in PAIs turned out to be crucial to drive the adsorption maximum toward physiological values of surface charge density, where octanol is desorbed. The electrostatic effects in the interfacial region reflected the adsorption properties: the changes in the potential drop across the interfacial region as a function of the surface charge density, in the physiological range, were opposite in PAIs as compared with apolar aliphatic compounds, as exemplified by octanol. This peculiar electrostatic effect of PAIs has far-reaching relevance for the design of inducers with an adequate therapeutic index to be used in clinical trials.

INTRODUCTION

While it is now accepted that neoplastic transformation does not necessarily destroy the potential of cancer cells to differentiate (Marks and Rifkind, 1991), a variety of agents are actively being studied, that induce these cells to rescue their differentiation program, thus ending their malignant in vitro and in vivo behavior. Among these agents, a series of substances collectively named polar/apolar inducers (PAIs) are particularly effective on numerous normal and transformed cells. This appellative is justified by the fact that, to be active, the members of this class must possess two, or even better three or four uncharged polar groups of limited bulk connected by apolar chains of about six carbons, usually a hexamethylene chain (Reuben et al., 1980). However, there is no simple correlation between the strength of the dipole moment and the biological activity, so that a large dipole moment does not insure effectiveness (Reuben et al., 1980; Marks et al., 1989; Breslow et al., 1991).

The simplest of PAIs, historically related to the pioneering work of Charlotte Friend on the erythroid differentiation of murine erythroleukemia cells (MELC) (Friend et al., 1971), is dimethylsulfoxide (DMSO), while the most thoroughly and widely studied is hexamethylenebisacetamide (HMBA), a potent inducer of various types of normal and cancer cells (Reuben et al., 1980).

The mechanism of action of PAIs is still obscure, although it is substantially agreed that their target is the plasma membrane (see Arcangeli et al., 1987, and references therein). Recently we proposed a mechanism (Arcangeli et al., 1993) based on the alteration of the electrical potential produced by PAIs adsorption at the interface between the polarized membrane surface and the bathing solutions. This proposal was first suggested by the evidence that commitment to differentiation of MELC is triggered by DMSO and HMBA synergistically with cation addition to the culture medium, depending on valence but not on species of cations. This synergy was explained by assuming that PAIs and cations cooperate to generate similarly oriented changes in the electrical properties of the plasma membrane and by this means to modulate voltage-sensitive proteins signaling the cell commitment. This hypothesis allowed the identification of a common target for several very different chemical agents ranging from ions to neutral molecules. This target is a unique physical parameter, the transmembrane potential (φ_m).

The biological implications of this perspective are relevant. In fact, modifications of the potential across either the internal or external membrane interface may influence the complex electrostatic phenomena occurring at these interfaces, such as ligand-receptor interactions and membrane activation of key components of cell signaling such as, e.g., protein kinase C (Mosior and McLaughlin, 1991). However, studies on merely the biophysical aspect of these signaling processes are difficult to perform at the cell membrane level or even on phospholipid bilayers because of the unavoidable complexity of chemical interactions of organic compounds with these structures.

To elucidate the purely interfacial effects of PAIs we then decided to study their adsorption and electrostatic effects in a physical apparatus, whereby these effects could be precisely measured at the surface of a charged mercury electrode, providing a clean and reproducible charged surface.

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Mercury is almost ideal from this point of view; in fact, the driving force responsible for adsorption is mainly the solute-solvent interaction (the so-called hydrophobic repulsion), that behaves independently of the nature of the charged surface (physisorption). Specific interactions between the solute and the adsorbing surface (chemisorption) are not relevant on mercury in the case of aliphatic compounds (Bockris and Reddy, 1970). Hence, data obtained on mercury allow us to single out and study, apart from the complexity of the full biological system, the mechanism of interaction of PAIs with the electrical charge distributed on a surrogate for the cellular membrane.

This approach enabled us to unravel the activity of the PAIs at charged interfaces, disclosing a structure-function relationship that may be responsible for their biological properties.

MATERIALS AND METHODS

Interfacial tension (γ) was measured at the mercury electrode-electrolytic solution interface, as a function of electrode polarization (φ) and PAI concentration (c). KCl and NaF at the fixed concentration 0.1 mol/L were used as the base electrolytic solution; varying amounts of the organic solutes were added. A set of electrocapillary curves was obtained measuring γ versus φ and c for HMBA, DMSO, and octanol in 0.1 M KCl; a further set was measured for HMBA in 0.1 M NaF. Data for 1-octanol in NaF were taken from a previous study (Carlà et al., 1989). Measurements, initially performed using 0.1 M KCl as the base electrolytic solution, were repeated in 0.1 M NaF to make certain that the adsorption behavior found in the presence of the Cl^-, just as in physiological media, was not influenced by the interference of PAIs with the anion adsorption to the electrode. In fact, whereas Cl^- sensibly adsorbs even at low negative values of surface charge density (Grahame and Parsons, 1961), F^- behaves almost ideally, showing very little specific adsorption (Grahame, 1954; Carlà et al., 1986) on a wide range of surface charge density.

Measurements have been performed with an experimental apparatus, described in detail in Carlà et al. (1991), based on the drop-shape technique; γ was obtained from the shape of an axysimmetric drop of mercury immersed in the electrolytic solution. The drop profile, taken through a solid-state video camera, was digitized, stored in a frame buffer and analyzed by an on-line computer μVAX 3500.

Reagents were KCl and NaF from Merck (Darmstadt, Germany), Suprapur grade; HMBA from Aldrich Chemical Co. (Milwaukee, WI) (purity 98%); DMSO from Sigma Chemical Co. (St. Louis, MO) (purity 99.9%) and Merck (Uvasol); octanol from Fluka (Buchs, Switzerland), (purity 99.5%). All reagents have been used without any further purification. Water was from a Millipore Milli-Q system (Millipore Corp., Boston, MA), fed with singly distilled spring water. Mercury was purified by repeated filtering and washing with concentrated HNO_3 and NaOH. The interface polarization was controlled by a potentiostatic circuit in a three-electrode configuration. Reference electrode was an Ag/AgCl electrode (manufactured by ECD, Florence, Italy) when the supporting solution was 0.1 M KCl and an ion-specific fluoride electrode (Metrohm (Zurich, Switzerland) EA 306-F) when the supporting solution was 0.1 M NaF.

Measurements were performed with the mercury polarization potential ranging from 0 to −1600 mV versus the reference electrode, with a 25 mV step. Accuracy of the polarization potential was better than ±0.3 mV. Concentration of the organic solute was varied in logarithmic increments over two to three decades, with eight steps per decade. Each set of electrocapillary curves, e.g., a scan over the full potential and concentration range, was obtained in a single run that lasted ~24–48 hours. Each experimental point was obtained according to the procedure described in Bordi and Carlà (1993). Experimental error on interfacial tension data contains two contributions: a random error $\epsilon_1 \simeq 0.15$ mJ/m^2 on each data point, mainly because of the process of digitizing the drop image; and an error $\epsilon_2 \simeq 0.6$ mJ/m^2 that comes from the calibration of the optical assembly and that is systematic inside a whole run depending upon just one such calibration. Only error ϵ_1 is relevant in data elaboration, as all thermodynamic quantities of interest in this work are obtained through differentiation of experimental data inside each single run, starting from surface charge density $\sigma_M = -(\partial\gamma/\partial\varphi)_c$ and surface excess $\Gamma = -(\partial\gamma/\partial RT \ln c)_\varphi$ (Mohilner, 1966). In the latter computation it has been assumed that the activity coefficient for the neutral solute is unity, as is usual with highly diluted solutions. Only in the case of DMSO at the highest concentration is this approximation rather poor, so that part of the data have to be regarded as qualitative only. All measurements were performed at temperature $T = 25 \pm 0.2$°C.

In all calculations we used the common assumption that at constant σ_M the Gouy-Chapmann ionic cloud is not significantly affected by the adsorption of aliphatic compounds, so that all the changes in the measured potential φ only affect the potential Δ_χ across first water monolayer in contact with the surface (compact layer).

RESULTS AND DISCUSSION

Peculiarities of the behavior of PAIs at interface were sought by using as a reference a typical aliphatic compound, 1-octanol. The latter is very similar to HMBA for the hydrophobic moiety (the hexamethylene chain), but substantially differs in its dipolar moment, being devoid of the strong polar acetamide groups placed at the ends of HMBA molecule (see formulas in Fig. 4 *A*). When tested on MELC, octanol was found to be devoid of any differentiating activity.

Adsorption as a function of σ_M relative to octanol is illustrated in Fig. 1 *A*, exemplifying the general behavior of aliphatic compounds; at different bulk concentrations, adsorption $\Gamma(\sigma_M, \ln c)$ is represented by bell-shaped curves, with the maximum near to the point of zero charge (pzc) and a rapid decline with σ_M increasing to either negative or positive values. This trend is explained by the following dielectric model (Devanathan and Tilak, 1965). Near the pzc, the electrical field in the interfacial region is zero (or very small) so that adsorption of organic solutes is sustained exclusively by the hydrophobic repulsion, driving solutes out of the solution bulk; as σ_M, and consequently the electrical field, increases toward negative or positive values, the component of the solution endowed with the higher dielectric constant (in this case water) is sucked toward the region of higher field intensity at the expense of the component with lower dielectric constant (in this case octanol), which is displaced from the surface. The non-exact coincidence of the adsorption maximum with the pzc is usually explained by the presence of a certain degree of spontaneous polarization of water in the compact layer even at $\sigma_M = 0$. This polarization, which slightly counteracts the octanol insertion into the compact layer, is the consequence of asymmetries of intermolecular interactions (mainly directional hydrogen bonds), which preferentially orient the negative end (oxygen) of the water electrical dipole toward the metal (Guidelli and Aloisi, 1992a).

When compared with octanol, HMBA displayed a strikingly different trend of interfacial adsorption (Fig. 1 *B*), with a marked shift of the maximum toward largely negative values of σ_M along with the concentration increase. A similar shift was displayed by DMSO (Fig. 1 *C*), a compound that, although chemically different, shares with HMBA and other

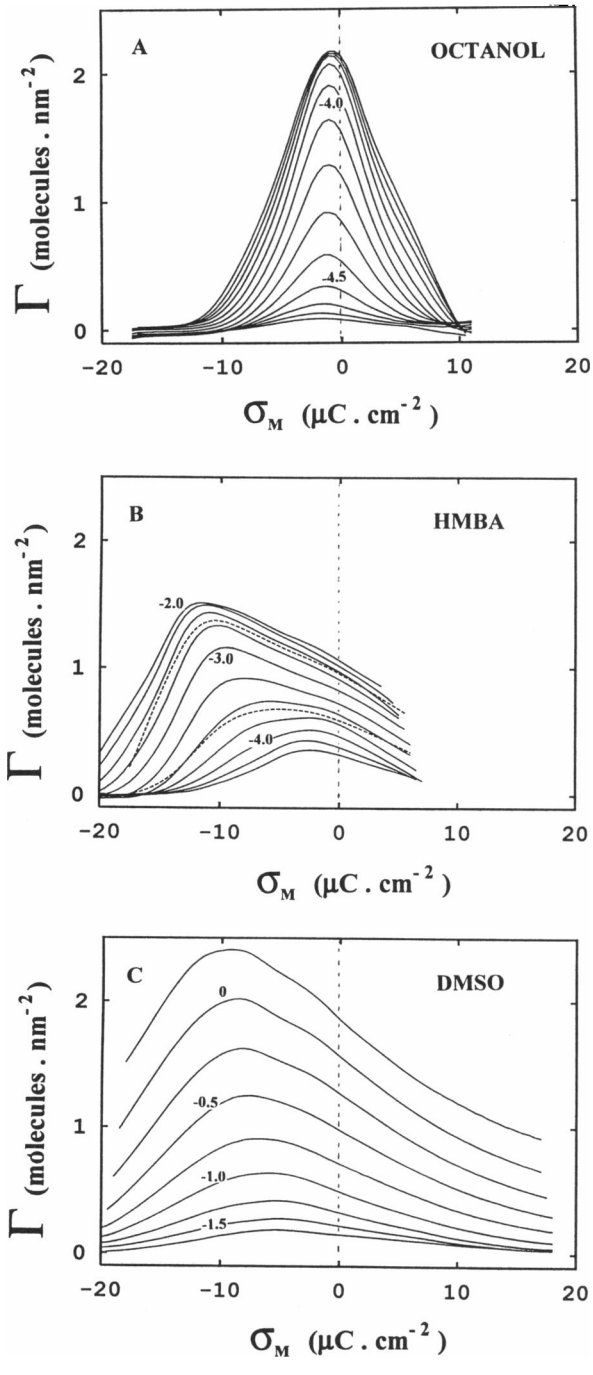

FIGURE 1 Adsorption properties of octanol (*A*), HMBA (*B*), and DMSO (*C*). Surface excess Γ, measured as indicated in Materials and Methods, is reported as a function of the electrode surface charge density σ_M for the following molar concentrations: (*A*) from $10^{-4.8}$ to $10^{-3.6}$ mol/kg, with $10^{0.1}$ steps; (*B*) from $10^{-4.5}$ to $10^{-2.0}$ with $10^{0.25}$ steps; (*C*) from $10^{-1.75}$ to $10^{0.25}$ with $10^{0.25}$ steps. Numbers close to the lines are decimal logarithm of concentration. Measurements were carried out using 0.1 M NaF as the base electrolyte. Notice that very similar adsorption curves were obtained both in NaF and KCl, excluding any contribution from a specific interference between anion and HMBA adsorption at the electrode. (*Dashed lines* in *B* refer to concentrations $10^{-3.5}$ and $10^{-3.0}$ in KCl).

PAIs the combination of polar and apolar groups. Because of this shift, which is clearly due to the high molecular dipoles, for the biologically effective concentrations of PAIs (5–10 mM for HMBA and ≃300 mM for DMSO), the maximum adsorption occurs at $\sigma_M \simeq -10$ μC/cm², i.e., in the range of the physiological charge density of cell membranes (McLaughlin, 1977; Gilbert and Ehrenstein, 1984; Tsong and Astumian, 1988).

Adsorption parameters for octanol and PAIs are reported in Table 1; notice that for octanol and HMBA surface molecular area at saturation is in agreement with the area obtained by computer molecular modeling, allowing the conclusion that both compounds adsorb flat at the electrode surface, displacing 9 and 13 water molecules, respectively. This is well in keeping with the report by Breslow et al. (1991) that, although flexible, each HMBA-type inducer binds to surface in an extended conformation.

The effects produced by adsorption of octanol and PAIs on the interfacial electrical potential are reported in Fig. 2, *A–C*. Values are expressed as the difference $\Delta_\chi - \Delta_{\chi 0}$, a function of σ_M, where Δ_χ and $\Delta_{\chi 0}$ are the potential drops across the compact layer in the presence and in the absence of the solute, respectively (see Fig. 4). In the negative range of σ_M, the maximum effect produced by octanol on $\Delta_\chi - \Delta_{\chi 0}$ is at −4 μC/cm², decreasing rapidly as σ_M approaches −10 μC/cm². On the contrary, HMBA and DMSO have their maximum effect at $\sigma_M = -14$ μC/cm², just where they get strongly adsorbed. Thus, between −5 and −15 μC/cm² the slope of $\Delta_\chi - \Delta_{\chi 0}$ versus σ_M is opposite for PAIs as compared with octanol. This fact should be kept in mind to understand the biological effects of these compounds (see below).

The potential curves reported in Fig. 2 are interpretable as follows. Each organic molecular species replacing water in the compact layer has its own polarizability in the electrical field generated by the surface charge; hence solute adsorption produces a change in the electrical potential across that

TABLE 1 Adsorption and molecular properties of PAIs as compared with octanol

Parameters	HMBA	1-Octanol	DMSO	H_2O
Γ_{MAX} (molecules·nm^{-2})	1.51	2.18	4.14	
Area/molecule (= $1/\Gamma_{MAX}$)/nm²	0.66	0.46	0.24	
Molecules of water displaced	13	9	4.7	
Molecular dipole moment ($C{\cdot}m{\cdot}10^{-30}$)	2·12.7	5.0	13.0	6.14
Area/molecule (nm^2) (from computer modeling)	0.60	0.47	0.22	0.051
Molecular dipole moment per unit area ($C{\cdot}m{\cdot}nm^{-2}{\cdot}10^{-30}$)	38.4	10.9	54	120

Values of maximal molecular adsorption Γ_{MAX} correspond to the point of adsorption saturation, i.e., the condition where the surface is totally covered by the solute and adsorption stops increasing with the solute concentration. For HMBA and octanol these data were obtained directly from data shown in Fig. 1; Γ_{MAX} for DMSO could not be obtained this way, as saturation was never reached. Instead, it has been computed as $[N_A d/m.w.]^{2/3}$, where d is density, *m.w.* is molecular weight and N_A is Avogadro's number. The program Hyperchem was used to compute dipolar moments and molecular dimensions. The area per molecule was obtained as the plane projection of the Van der Waals surface with the molecule lying with its major axis parallel to the plane.

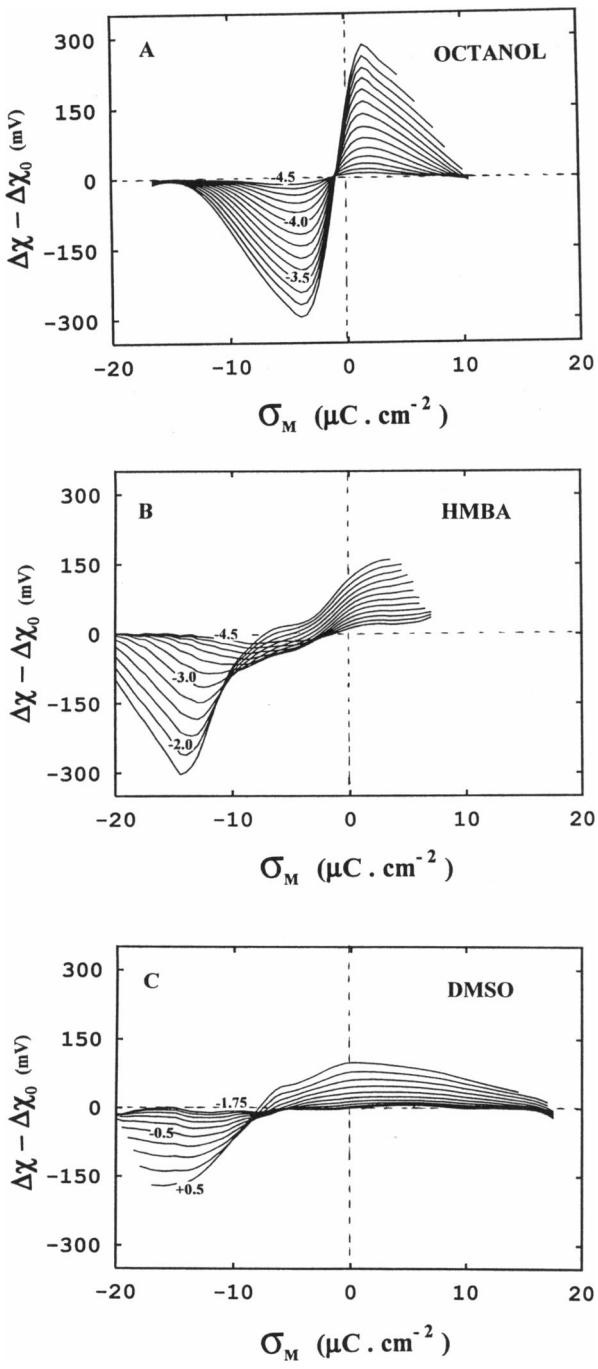

FIGURE 2 Shift of the electrical potential across the compact layer ($\Delta_\chi - \Delta_{\chi 0}$) as a function of σ_M and solute concentration c. Labels and concentration steps are the same as in Fig. 1. $\Delta_\chi - \Delta_{\chi 0}$ has been obtained subtracting, from the polarization potential $\varphi(\sigma_M,c)$ of the measuring cell, the potential $\varphi(\sigma_M,0)$, i.e., the potential at the same surface charge density σ_M, with no solute. In fact, at constant σ_M and varying the concentration of a neutral solute that adsorbs in the compact layer, all changes in the cell potential reflects in identical changes across the compact layer. Notice that in the physiological range of σ_M ($\simeq -10$ $\mu C/cm^2$) the slope of the curves is opposite for PAIs as compared with octanol.

layer, $\Delta_\chi - \Delta_{\chi 0}$. Because of the water spontaneous polarization at $\sigma_M = 0$, a significant change occurs also at pzc. When the charging of the electrode surface strengthens the electrical field toward either negative or positive values, water polarization is enhanced accordingly, so that replacement by solute produces a larger effect on $\Delta_\chi - \Delta_{\chi 0}$. However, at a certain value of σ_M, the field becomes strong enough to determine the progressive solute desorption and eventually $\Delta_\chi - \Delta_{\chi 0}$ reverts to 0. In the case of HMBA and DMSO, the shift of maximum adsorption toward negative values of σ_M moves in the same direction the maximum effect on $\Delta_\chi - \Delta_{\chi 0}$ (cfr. Figs. 1 and 2).

The nature of the forces implicated in this shift were clarified parametrizing adsorption data by Frumkin's isotherm as reported in Fig. 3. This procedure allowed the calculation of

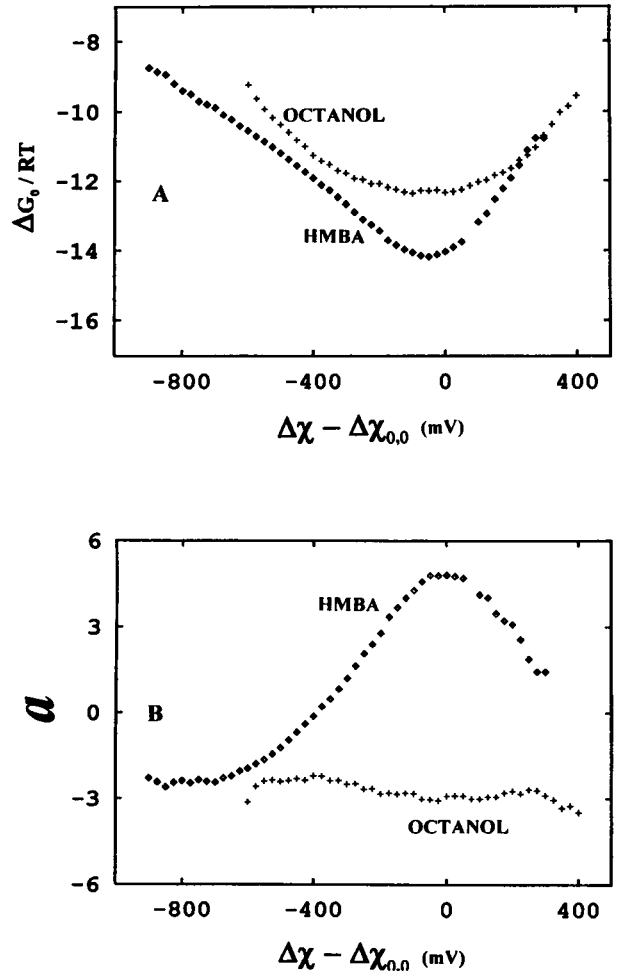

FIGURE 3 Adsorption parameters for HMBA ◇ as compared with octanol (+). Adsorption data of Fig. 1 have been fitted according to the Frumkin's isotherm $\ln[m(1-\theta)/\theta] = -(\Delta G_o/RT) - a\theta$, where m represents the mole ratio in the solution (number of mol solute)/(number of mol solvent) and $\theta = \Gamma/\Gamma_{MAX}$. ΔG_0, a and Γ_{MAX} are three adjustable parameters that represent, respectively, the free energy of adsorption (i.e., the change in the free energy of the system when unitary amount of substance passes from the solute to the adsorbed state, in the limit of very low adsorption $\theta \rightarrow 0$); the so-called Frumkin interaction factor, which describes the dependence of the free energy of adsorption against θ; the maximum adsorption as defined in Table 1. Plot of $\ln[m(1-\theta)/\theta]$ versus θ at constant potential $\Delta_\chi - \Delta_{\chi 0,0}$ gave straight lines showing that a is a constant ($\Delta_{\chi 0,0}$ is the potential across the compact layer at pzc and solute concentration equal to 0 (plot not shown). The slope and intercept with ordinates gave a and ΔG_0, respectively, reported in A and B as a function of $\Delta_\chi - \Delta_{\chi 0,0}$. ΔG_0 is reported as $\Delta G_0/RT$.

the free energy of adsorption (ΔG_0), i.e., the change in the free energy of the system when a unitary amount of the solute gets adsorbed in the compact layer, in the limit of very low adsorption, which is when the effects of interaction among adsorbed molecules are negligible. As shown in Fig. 3 *A*, minimum ΔG_0 was $-12.4\,RT$ (where R is the gas constant and T is the thermodynamic temperature) at −70 mV for octanol and −14.2 RT at −60 mV for HMBA, showing that both compounds have a very similar tendency to get adsorbed near the pzc. The slightly lower ΔG_0 value for HMBA is consistent with the presence in this molecule of a second hydrophobic $-CH_3$ head, whereas the acetamide groups give only a small contribution in accordance with their high dipole moment. From these data it can be concluded that the PAIs' characteristic shift of maximum adsorption to largely negative values of σ_M cannot be ascribed to a direct interaction of the adsorbed dipoles with the electrical field, as this would imply a corresponding shift of ΔG_0; instead, it is explained by data in Fig. 3 *B*, illustrating the so-called Frumkin interaction factor a. The latter summarizes all changes in ΔG_0 when solute molecules get adsorbed interacting with each other. In the case of octanol, we found an almost constant value $a \simeq -3$, which corresponds to an attractive interaction among adsorbed molecules. This finding, usual for aliphatic compounds, is due to the fact that the number of water-water bonds, which must be broken to allow the solute adsorption, progressively diminishes with the number increase of previously adsorbed molecules (Guidelli and Aloisi, 1992b). For HMBA we found a positive (repulsive) value of a near the pzc, which gradually decreases and eventually reaches the attractive value of $\simeq -3$ at extreme negative polarization of the compact layer.

Evidently, adsorption of HMBA in the compact layer tends to be impaired by a sort of intermolecular repulsion, which is maximal at pzc and disappears at highly negative values of σ_M. This effect is attributable to the fact that the electrical dipoles of HMBA are strong enough to link a hydration shell made up of several water molecules (see Fig. 4). This shell has maximal dimension at $\sigma_M = 0$, being progressively stripped off when σ_M increases, polarizing the water in the compact layer. Thus the encumbrance of HMBA molecules decreases with the increase of the interfacial electrical field; this allows a larger adsorption up to the point at which solute desorption, produced by water polarization, prevails.

CONCLUSIONS

Interfacial adsorption of PAIs, as has emerged from this study, strikingly differs from that so far described for apolar aliphatic compounds. This difference essentially consists in

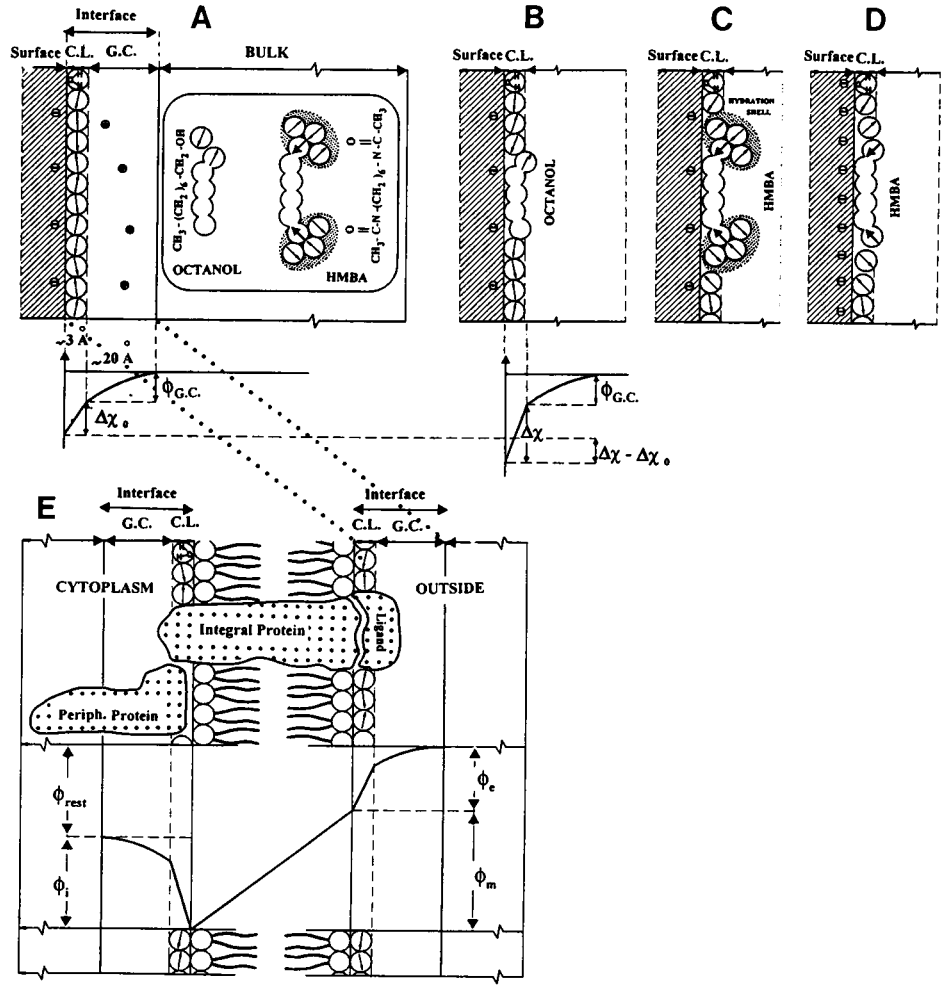

FIGURE 4 Schematic representation of the interfacial adsorption of PAIs and octanol. (*A*) Outline of the interfacial region. (Inset) Chemical formulas of octanol and HMBA. Arrows represent the electrical dipoles. Notice the hydration shells surrounding the polar heads. The diagram below illustrates the electrical potential across the compact layer with no solute adsorbed ($\Delta_{\chi 0}$) and across the Gouy-Chapmann layer (φ_{GC}). (*B*) Adsorption of octanol in the compact layer. Water substitution by octanol alters the potential across the compact layer. Values of $\Delta_\chi - \Delta_{\chi 0}$ are reported in Fig. 2 *A*. (*C*) Adsorption of HMBA in the compact layer. Values of $\Delta_\chi - \Delta_{\chi 0}$ are reported in Fig. 2 *B*. (*D*) The HMBA molecule has lost its hydration shell after the increase of the negative surface charge. (*E*) Model to represent a cell plasma membrane and its internal and external interfacial regions. The structure outlined in Fig. 4 *A* appears twice in this model, at both sides of the membrane. Combined effects on the internal and external Δ_χ produce the change in φ_m responsible for the electrostatic modulation of membrane proteins. *Abbreviations:* Surface, charged surface; C.L., compact layer; G.C., Gouy-Chapmann diffuse layer; φ_m, transmembrane potential; φ_i,φ_e, potential across the internal and external compact and diffuse layer; φ_{REST}, resting potential of the membrane.

the PAIs capability to get maximally adsorbed at physiological charge densities for cell membranes ($\sigma_M \simeq -10\ \mu C/cm^2$), where aliphatic compounds are nearly completely desorbed (Fig. 1). Because of such a difference, although in the above σ_M range both PAIs and apolar aliphatic compounds produce a negative shift of the potential across the interfacial region, the slope of the shift versus σ_M is opposite (Fig. 2).

While identifying the merely physical behavior of PAIs at a charged interface, these findings can be extrapolated to cellular models, keeping in mind the picture outlined in Fig. 4 *E*. Biological signals such as, e.g., stimuli committing MELC to differentiation, are canonically conceived as transduced by membrane proteins, either integral (spanning across the membrane) or peripheral (activating at the internal interface of the plasma membrane). These proteins are subject to undergoing conformational changes upon variation of the electrical field inside or at the surface of the membrane (Tsong and Astumian, 1987, 1988; Honig et al., 1986). In the MELC model, our discovery that the potential shift produced by cations in the external Gouy-Chapmann region of the plasma membrane cooperates with the cell commitment indicated that the putative signaling proteins were activated by the negative shift of the electrical field inside the membrane; this corresponds to a hyperpolarization (Arcangeli et al., 1993). The present study points out that, at the physiological σ_M values, adsorption of neutral organic molecule in the compact layer at the external cell surface invariably produces an opposite shift. Thus the first effect of this adsorption at the external surface of a cell is expected to be a strong depolarization of the plasma membrane, accounting for an "electrostatic toxicity" of the compounds, to be added to their chemical toxicity.

However, whenever a compound penetrates the cell, it acts on both sides of the membrane. This was shown for HMBA, which equilibrates across the plasma membrane within ~6 h after addition to the culture (Reuben et al., 1980). Under these conditions, the net effect on the transmembrane potential φ_m is given by the difference between the internal and external effects on the corresponding potential (φ_i,φ_e), eventually depending on the difference between the internal and external σ_M. If, as in the case of MELC, this shift has to be negative, one should conclude that the internal charge is higher than the external one. In any case, the resulting potential shift will be proportional to the slope of the curve representing the potential drop across the compact layer versus σ_M (Fig. 2). As mentioned above, this slope is strictly conditioned by the behavior of molecular adsorption at interface as a function of σ_M and is just what distinguishes PAIs from apolar aliphatic compounds. Since the slope is opposite for PAIs as compared with octanol, one should expect equally opposite biological effects. As a matter of fact, when tested on MELC, octanol antagonized the cell commitment to differentiation operated by HMBA (data not shown).

An important point to stress here is that the peculiarity of the activity of PAIs at interface stems from exactly the same structural features identified as necessary for their biological effectiveness by the thorough analysis performed by the group of Marks and Rifkind (Reuben et al., 1980; Marks et al., 1989; Breslow et al., 1991). In fact, the requirements to have a good inducer (two or more polar groups separated by a flexible polymethylene chain of five to six methylene groups) have now an explanation in the mechanism underlying the production of the potential shift at the interface. In fact, in the case of a compound similar to HMBA but with a longer apolar chain, the hydrophobic adsorption of the molecule would tend to increase, but the crucial effects of the polar heads, necessary to shift adsorption toward a physiological σ_M value, would be diluted in a too-large area, with a smaller overall effect. On the contrary, a shorter chain would impair the interfacial adsorption of molecules endowed with a high dipole moment. There is an optimum compromise between dipolar moment and hydrophobicity that affords the maximal interfacial effect, as well as the maximal biological activity. In this respect, it is important to note that in HMBA and DMSO the ratio among dipolar moment and occupied area is roughly the same and is very similar to that of most compounds displaying a good inducing activity (Reuben et al., 1980; Marks et al., 1989; Breslow et al., 1991). In this light it is also easy to explain why one can change the architecture of the HMBA molecule dimerizing acetamide by linking the methyl groups, obtaining a compound (suberic acid bis(*N*-methylamide), SBDA: CH_3—N—CO—$(CH_2)_6$—CO—N—CH_3) that has the same activity as inducer to HMBA. The latter and SBDA have their polar groups separated by identical methylene bridges and they have a similar ratio of polar to apolar hydrophobic moieties (Breslow et al., 1991).

To conclude, it is worth noting that, in cell models similar to that proposed for MELC, the charge imbalance between internal and external surface is critical for determining the magnitude and the sign of the potential shift and the relative effects. In cells whose plasma membrane has a charge imbalance opposite to that supposed in MELC, one should expect that PAIs antagonize instead of promoting differentiation, whereas ordinary aliphatic compounds could turn out to act as effective inducers. This makes it difficult to predict the inducing efficacy of PAIs from one cell type to another. Moreover the charge density of the plasma membrane is a parameter that changes in various biological processes, including differentiation and neoplastic transformation (Brown et al., 1979; Becchetti et al., 1992). Within these limits, elucidation of interfacial effects of PAIs should provide far-reaching contributions to design inducers endowed with sufficient therapeutical index to be used in clinical trials (Marks et al., 1994).

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