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Surface Activities of Procaine, Lidocaine, and Tetracaine and Their Interaction Energies with Phospholipid Monolayers

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Abstract □ The free energies of adsorption of procaine, lidocaine, and tetracaine at the air–water interface were estimated from plots of surface pressure ($\pi \leq 5$ dynes/cm) against bulk concentration. Their interaction energies with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers, previously spread at the air–water interface, were estimated from the increase of surface pressure with increasing concentrations of the subphase-injected anesthetic. Free energies of adsorption and the interaction energies were in the order procaine < lidocaine < tetracaine and correlate with relative anesthetic potencies and the blocking of nerve conduction.

Keyphrases □ Anesthetics, local—free energies of adsorption at air–water interface and interaction energies with phospholipid monolayers correlated with pharmacological activity □ Adsorption, free energies—various local anesthetics at air–water interface, correlated with pharmacological activity □ Interaction energies—various local anesthetics with phospholipid monolayers, correlated with pharmacological activity □ Phospholipid monolayers—interaction energies with various local anesthetics correlated with pharmacological activity □ Surface activities—various local anesthetics, correlated with pharmacological activity

The correlation between the potency of local anesthetics in blocking nerve conduction and their penetrations into lipidic monolayers has been demonstrated (1–5). More recently, it was shown by NMR (6, 7) and spin-labeled local anesthetics (8) that they do penetrate into zwitterionic phospholipid bilayers of liposomes.

The present work examines the surface activities of procaine, lidocaine, and tetracaine at the air–aqueous interface and estimates their interaction energies with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers, previously spread at the air–aqueous interface.

EXPERIMENTAL

Reagents—Procaine hydrochloride[1], lidocaine hydrochloride[2], and tetracaine hydrochloride[2] were used without further purification. Dipalmitoyllecithin[3], dipalmitoylphosphatidylethanolamine[4], hexane[5] used for the preparation of the spreading solutions, and distilled water used for the preparation of the solutions fulfilled the requirements previously specified (9, 10). Analytical reagent sodium chloride[6] was roasted for 6 hr at 700° prior to the preparation of the aqueous solutions to remove surface-active organic impurities.

Instruments and Methods—Surface tension was measured with a Wilhelmy platinum plate attached to an electrobalance[7] whose output was fed into a recorder[8]. The methods for the measurement of the surface tension of aqueous solutions, γ, and the change of the surface pressure, $\Delta\pi$, of the phospholipid monolayer as a function of time after drug injection in the subphase were described previously (9–11). All experiments were performed in 0.15 *M* NaCl at 20 ± 1°.

In the injection experiments, the initial surface pressure of the phospholipid monolayer spread at the air–0.15 *M* NaCl interface was 5 ± 0.1 dynes/cm. The surface pressure, π, of the drug solution is the difference between the previously determined surface tension of the 0.15 *M* NaCl, γ_{NaCl}, and the surface tension of the drug solution in this saline solution, γ_d. The surface pressure, π, was fitted to a function of the drug concentration, *C*, by digital computerized nonlinear regression to exponential equations of the form:

$$\pi = \gamma_{NaCl} - \gamma_d = B_1 e^{[B_2(\log C)^2 + B_3 \log C]} \qquad \text{(Eq. 1)}$$

where the B_i values were adjustable parameters (9).

Densities of the drug solutions were determined with 10-ml specific gravity bottles previously calibrated with water. The precision of the weighing was ± 0.1 mg.

RESULTS

Adsorption at Air–Aqueous Interface—Typical plots of the surface pressure against the logarithm of the concentration (moles per liter) for procaine hydrochloride, lidocaine hydrochloride, and tetracaine hydrochloride in 0.15 *M* NaCl are given in Fig. 1.

A simple expression for the free energy of adsorption was derived (12) from thermodynamic and molecular kinetic considerations:

$$\Delta G^\circ = -RT \ln \frac{\pi}{X_2^*} \qquad \text{(Eq. 2)}$$

where ΔG° is the change in standard free energy associated with the adsorption of the solute at an air–water interface, π is the surface pressure, X_2^* is the activity of the solute, and R and T are the universal gas constant and the absolute temperature, respectively. Thus, the numerical value of ΔG can be calculated from the slope, π/X_2^*, of a linear plot of π against the mole fraction, X_2, of the solute in bulk solution when $X_2 \to 0$ and $X_2 \to X_2^*$ at low mole fractions. This expression was used recently for the estimation of the free energy of adsorption of alkanols from C_1 to C_{14} at the air–aqueous interface (11).

The plots (Fig. 2) of π against X_2 for the three compounds were reasonably linear in the region $\pi \leq 5$ dynes/cm under the experimental

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Pfaltz and Bauer, Stamford, Conn.
[3] Applied Science Laboratories, State College, Pa.
[4] Schwarz-Mann Research Laboratories, Orangeburg, N.Y.
[5] J. H. Baker Chemical Co., Phillipsburg, N.J.
[6] Mallinckrodt Chemical Works, St. Louis, Mo.
[7] Cahn Division, Ventron Instrumental Corp., Paramount, Calif.
[8] Leeds Northrup, North Wales, Pa.

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Table I—Free Energies of Adsorption at the Air-Water Interface (ΔG), Interaction Energies with Phospholipid Monolayers (Ψ), and Free Energies of Transfer (ΔG_{tr}) from Water to Octanol of Procaine, Lidocaine, and Tetracaine

Local Anesthetic	Interface: Air–0.15 *M* NaCl, $\Delta G°$, kcal/mole	Interface: I[a]–0.15 *M* NaCl, Ψ, kcal/mole	Interface: II[b]–0.15 *M* NaCl, Ψ, kcal/mole	Octanol-Water[c], $\Delta G°_{tr}$, kcal/mole	Relative[a] Anesthetic Potency	Relative[e] Blocking Potency
Procaine	3.9	3.5	4.2	2.5	1	1
Lidocaine	4.9	3.6	5.1	—	3.8	—
Tetracaine	7.3	6.5	6.5	5.0	36.5	460

[a] I = dipalmitoyllecithin monolayer. [b] II = dipalmitoylphosphatidylethanolamine monolayer. [c] Calculated from Ref. 17. [d] Reference 2. [e] Reference 13.

conditions. The intercepts were close to the limits of the reproducibility of the surface tension measurements (±0.2 dyne/cm). The estimated values of $\Delta G°$ (±0.2 kcal/mole) from such plots are given in Table I. The free energies of adsorption, $\Delta G°$ (kilocalories per mole), were in the order tetracaine > lidocaine > procaine and correlate with their blocking potencies on the decapitated frog sciatic nerve (2) and their anesthetic potencies obtained with the frog sciatic nerve trunk (13).

Interaction with Phospholipid Monolayers—The interaction of the subphase-injected drugs with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers was virtually complete in the first 15 min after the injection. The criterion of equilibrium was the constancy (±0.1 dyne/cm) of the surface pressure increment, $\Delta\pi$, during 30 min. The interaction energies, Ψ, were estimated (9–11, 14) from the slopes of the linear plots of the reciprocals of the equilibrium surface pressures, $\Delta\pi_{eq}$, after the injection against the reciprocals of the final concentrations, n (molecules per cubic centimeter), of the subphase-injected drug. In both cases, such energies (Table I) were in the order procaine < lidocaine < tetracaine.

DISCUSSION

Adsorption at Air–Aqueous Interface—Even for nonideal solutions, the amount of surfactant ion present in an adsorbed monolayer at the air–aqueous interface can be estimated directly from measurements of the variation of the surface tension with surfactant bulk concentration at constant counterion concentration using the equation:

$$-\Gamma_2^m = \frac{1}{RT}\left(\frac{d\sigma}{d\ln m_2}\right)_{m_3} \qquad \text{(Eq. 3)}$$

where Γ_2^m is the amount of surfactant ion in the monolayer (moles per square centimeter), R is the universal gas constant (ergs per degree per mole), T is the absolute temperature (degrees Kelvin), σ is the surface tension of the surfactant solution (dynes per centimeter), and m_2 and m_3 are the molalities (moles per kilogram) of the surfactant ion and of the counterion, respectively, in the bulk solution (15).

Equation 3 can be conveniently transformed into:

$$\Gamma_2^m = \frac{1}{RT}\left(\frac{d\pi}{d\ln m_2}\right)_{m_3} \qquad \text{(Eq. 4)}$$

where π is the surface pressure (dynes per centimeter) of the surfactant solution containing a constant (m_3) molal counterion concentration.

The derivative $[d\pi/(d\ln C_2)]$ of the exponential equation (Eq. 1) that characterized the dependence of the surface pressure on the concentration was computed with respect to the logarithm of the concentration (moles per kilogram) of the anesthetic compound. Substitution of these values into Eq. 4 permitted the calculation of the amount of anesthetic ion in the monolayer, Γ_2^m (moles per square centimeter), at any bulk concentration.

Space-filling molecular models and molecular volume calculations performed using group increments (16) indicate that the molecular dimensions of these anesthetic ions are such that they can be accommodated into a thin interfacial region 10–20 Å thick. On the premise that the anesthetic ions that form the adsorbed monolayer at the air–aqueous interface are completely immersed in the aqueous phase and on the assumption of an average thickness of 15 Å, the volume, V_s, of the interfacial region containing the number of anesthetic ions, Γ_2^m (moles per square centimeter), can reasonably be estimated for comparative purposes.

Plots of the concentration of such a thin interfacial region [$\Gamma_2' = (\Gamma_2^m/V_s)$ in moles per liter] estimated from these volumes and from the surface concentration, Γ_2^m (moles per square centimeter), against the bulk concentration, C (moles per liter), are given in Fig. 3 for procaine, lidocaine, and tetracaine and indicate that the concentration of adsorbed tetracaine at the thin interfacial region could be up to 10^3 times greater than that of procaine or lidocaine with the same experimental conditions.

Interaction with Phospholipid Monolayers—Dipalmitoylphosphatidylethanolamine forms a condensed liquid monolayer. The strong $P^- \sim N^+$ electrostatic interaction of the zwitterionic hydrophilic polar groups jointly with the attractive forces between the hydrocarbon chains of neighboring molecules produce a rigid structuring where any net electrical charge is essentially negligible (14).

Dipalmitoyllecithin forms a relatively less condensed monolayer at the air–water interface, because the shielding effect of the positively charged amino group by the three methyl groups decreases the attractive potential between neighboring molecules (14).

Partition coefficients have been measured between octanol–aqueous solution for procaine (17) and tetracaine (18). On the assumptions that the given numerical values of the interaction energies of procaine and tetracaine are valid estimates (*i.e.*, the entropies are invariant) and that the same energies are operative under the conditions in which partition coefficients were measured, the changes in free energies that correspond

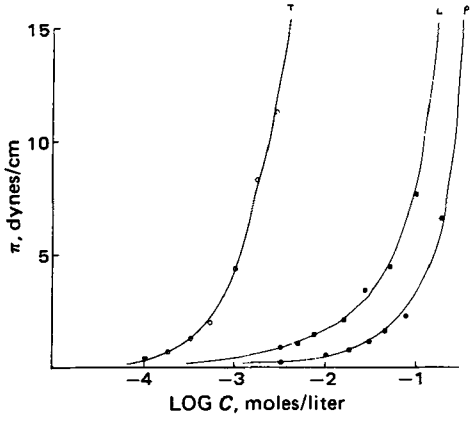

Figure 1—*Plots of surface pressure, π, against the logarithm of the bulk concentration, C (moles per liter), for tetracaine (T), lidocaine (L), and procaine (P). The lines drawn through the experimental points were the "best fit" to Eq. 1 obtained from the computer.*

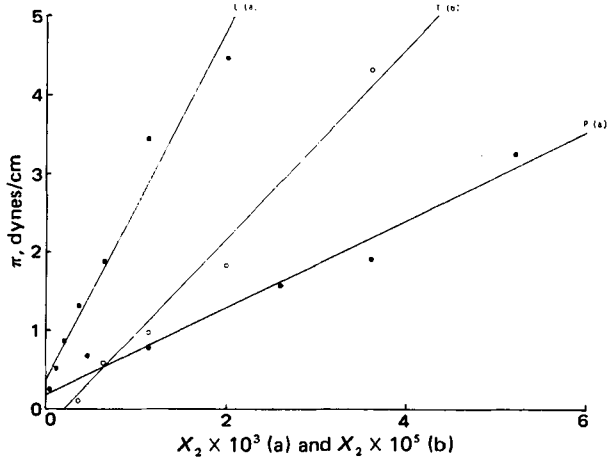

Figure 2—*Plots of surface pressure, π (≤5 dynes/cm), against the mole fraction, X_2, for tetracaine (T), lidocaine (L), and procaine (P).*

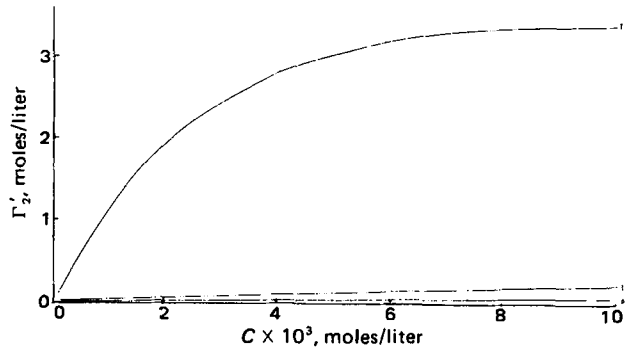

Figure 3—*Plots of concentration at the interfacial region* (Γ'_2, *moles per liter*) *against bulk concentration* (C, *moles per liter*) *for tetracaine* (*T*), *lidocaine*, (*L*), *and procaine* (*P*).

to octanol–water partition coefficients of procaine and tetracaine were calculated from the literature data (17) using:

$$\Delta G^\circ_{tr} = RT \ln P \qquad \text{(Eq. 5)}$$

where P is the partition coefficient and ΔG°_{tr} is the standard free energy of transfer of the solute. Such energies (ΔG°_{tr}) (Table I) correlate with the interaction energies with phospholipid monolayers.

NMR evidence for the hydrophobic interaction of tetracaine, lidocaine, and procaine with egg yolk phospholipid liposomes indicates that the aromatic protons of procaine are affected more drastically than those between the aromatic side of the molecule and the quaternary amine. The less affected protons are those of the CH_2N groups, which suggests that they face the aqueous phase. For tetracaine, the less affected protons are those of the CH_2N groups; the more affected are those of the butylene groups at the nonpolar side of the molecule. These findings indicate that the property of penetrating the liposome membrane is dependent on the nonpolar side of the molecules (6, 7).

The results show that the interaction energies of tetracaine with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers are higher than those of lidocaine and procaine. This fact seems to confirm that the enhanced hydrophobicity of the nonpolar end of this molecule due to the presence of the butylene group favors the penetration of the tetracaine nonpolar moiety through the liposome bilayers. This facilitated penetration and the observed increase of the relative anesthetic and blocking potencies may be the results of the comparatively greater interfacial concentration of tetracaine.

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