The Interaction of Local Anesthetics with Lecithin Monolayers

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SUMMARY

The pressure-area curves were determined for synthetic L-α-(dipalmitoyl) lecithin monolayers on subsolutions containing the minimum blocking concentrations of the local anesthetics β-naphthol thymol, ephedrine, procaine, tetracaine, phenyltoloxamine, quinine, and dibucaine. It is shown that there is an approximately linear relationship between the log of the rate of change of the surface pressure with concentration of these anesthetics and the product of the mole refraction and ionization potential. This correlation is related to a partition function based upon the London interaction energy.

INTRODUCTION

This paper is an extension of previous work (1) on techniques of investigating the action of local anesthetics. A semi-theoretical expression for the partition function for local anesthetics distributed between the cell membrane surface and the adjacent extracellular solution was presented. The relation is

$$\ln (\mathrm{MBC}) = \ln C_s - KR_0I \qquad (1)$$

where MBC is the minimum blocking concentration defined as the minimum concentration in the external solution necessary for block of excitability (1). C_s is the minimum blocking concentration of molecules at the surface, R_0 the mole refraction, and I the ionization potential. K is primarily a function of interaction distances and was considered as a first approximation to be a constant.

This approach is based upon the difference in the London interaction energy of the local anesthetic molecule in the aqueous solution and at the cell membrane surface.

There is evidence that phospholipid molecules play a role in biological membrane structure (2, 3). Therefore it seems appropriate to investigate the nature and extent of interaction between the local anesthetic and phospholipid molecules. One way of studying this interaction is by measuring the surface tension at the phospholipid monolayer/aqueous solution interface in the presence of local anesthetic molecules (4, 6).

In this work the pressure-area curves were determined for synthetic L-α-(dipalmitoyl) lecithin monolayers on subsolutions which contained 5 mM phosphate buffer, pH 6.8, and the minimum blocking concentration of 8 local anesthetics.

We wish to thank Dr. D. P. Agin for suggesting this problem to us and for assistance with the design of the experimental setup.

METHOD AND MATERIALS

A 1 mM solution of synthetic L-α-lecithin-(dipalmitoyl) in *n*-hexane/ethyl alcohol 4:1 (V:V) was quickly dripped onto a solution containing a 5 mM phosphate buffer, pH 6.8, and various concentrations of

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[2] The values for (MBC) and the product R_0I are taken from a previous paper (1). In the case of the anesthetics which have tertiary amine groups, (MBC) represents the concentration of the nonionized base.

local anesthetic. A Roger Gilmont precision microsyringe was used to dispense the monolayer solution. The subsolution was contained in a Teflon-coated aluminum surface tray (24.1 × 14.0 × 1.6 cm) and compressed with a Lucite barrier. The entire tray was immersed in a water bath at 25.3 ± 0.3° which had a Lucite cover.

The surface pressure was determined by the Wilhelmy plate method (7). The surface tensions of the anesthetic solutions without monolayer were measured by using the plate-detachment technique (8). A glass coverslip was suspended from a Statham Microscale accessory which was attached to a Statham universal transducing cell (Model UC2). The output of the cell was measured with a Keithley 149 millimicrovoltmeter. Surface areas were measured to 1%. Surface pressures were measured to ±0.05 dyne/cm at about 100% relative humidity. Calibration was achieved by adding known weights to the transducer.

Compression of the film was performed manually at a rate of about 4 Å² per molecule per minute. Reduction of the area was started after a steady value of the surface tension was noted. This usually occurred after 30 min to 1 hr. The value of the surface pressure was taken at the end of 2 min at each area. An increase in the surface tension with time was noted in most cases below areas of 60 Å² per molecule of lecithin. Any rate of increase greater than 0.5 dyne/cm min was regarded as "collapse" and the experiment was halted.

Each pressure-area curve presented in the Results section is the average of at least two measurements. The curve where the 5 mM phosphate solution was employed is the average of 6 experiments. The standard error is about ±0.05 dyne/cm for the phosphate solution and about ±0.3 dyne/cm for the anesthetic solutions.

The chemicals were reagent grade and were used without further purification. The synthetic lecithin (dipalmitoyl) was purchased from Mann Research Laboratory as a chromatographic standard. The water used was redistilled in a Pyrex® distillation unit.

RESULTS AND DISCUSSION

The pressure-area (π-A) curve for synthetic L-α-(dipalmitoyl)lecithin on a 5 mM phosphate buffer, pH 6.8, is consistent with the recent work of Shah and Schulman (9). This curve and the π-A plots for the same monolayer on solutions containing the MBC of 8 local anesthetics are shown in Fig. 1. We have plotted the surface pressure, π, against the area per molecule of lecithin. The surface pressure in these curves is defined as

$$\pi = \gamma - \gamma'$$

where γ is the surface tension of the 5 mM phosphate solution without the monolayer of lecithin and γ' is the value of the surface tension of the monolayer covered solution.

Figure 1 demonstrates the striking similarity of the π-A curve of each anesthetic. The MBC of the anesthetic lowers the surface tension of the lecithin/water interface by approximately the same amount, and the π-A curve follows a similar relation between the surface tension and surface area of the lecithin monolayer. This is in accord with the work of Skou (5). Skou's results cannot be quantitatively compared

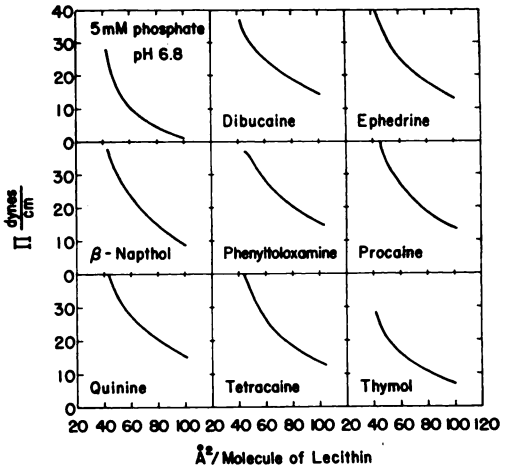

FIG. 1. *Plot of the surface pressure π of lecithin monolayers versus the area per molecule of lecithin on subsolutions containing the minimum blocking concentration (Table 1, column 4) of 8 local anesthetics with 5 mM phosphate buffer, pH 6.8, at 25.3 ± 0.3°*

to ours since he worked with a monolayer of unidentified lipids extracted from peripheral nerve. However, the changes in π that he noted are of the same order of magnitude and were also independent of the area of the monolayer.

These changes in surface tension can be dealt with in a semiquantitative manner by using the thermodynamic analysis of Skou (5) and the relationship between the MBC and the molecular parameters R_0 and I in Eq. (1). Only the change in π due to the addition of local anesthetic at a constant area per molecule of lecithin monolayer will be considered.

Following Skou's use of Gibbs' adsorption theorem in the case of a lipoid monolayer in equilibrium with an aqueous solution of the local anesthetic, we have

$$\Gamma = \frac{C}{RT}\frac{\partial \pi}{\partial C} \qquad (2)$$

where Γ is the apparent surface concentration of the local anesthetic in moles per square centimeter of surface, C is the concentration of the anesthetic in the subsolution in mM/l, π is the surface pressure in dynes/cm, R the gas constant, and T the absolute temperature. In the derivation of Eq. (2) the usual substitution of concentrations for activities is made and the term for the virtual change in the free energy of the insoluble monolayer is considered negligible.

By multiplying both sides of Eq. (2) by the factor d^{-1}, where d is some arbitrary depth of the surface phase, we have

$$C_s = \frac{\Gamma}{d} = \frac{C}{dRT}\frac{\partial \pi}{\partial C} \qquad (3)$$

where C_s represents the surface concentration of the local anesthetic in mM/l. This equation may be combined with Eq. (1) to obtain

$$\ln \frac{\partial \pi}{\partial C} = KR_0I + \ln dRT \qquad (4)$$

Since the minimum blocking concentrations of local anesthetic are generally quite low, we can approximate the term on the left in Eq. (4) with the relations

$$\ln \frac{\partial \pi}{\partial C} \cong \ln \frac{\Delta \pi}{\Delta C} = \ln \frac{\Delta \pi}{\text{MBC}} \qquad (4a)$$

$\Delta\pi$ is defined as

$$\Delta \pi = \pi^* - \pi = \gamma' - \gamma^*$$

where π and γ' refer to the monolayer on the 5 mM phosphate buffer alone. π^* and γ^* are the values of the surface pressure and tension, respectively, of the monolayer-phosphate buffer-local anesthetic system.

Assuming that all the gross approximations made so far are reasonable, Eq. (4) requires a linear relationship between ln $\partial\pi/\partial C$, which has been approximated by the ratio of increments ln $\Delta\pi$/MBC, with the product R_0I. For $\Delta\pi$ we consider the values of π and π^* at the initial area of 100 Å²/molecule of lecithin. These values of $\Delta\pi$ and those of the product R_0I and the log (MBC) are presented in Table 1.[2] Also included in Table 1, for comparison, are the values of $-\Delta\gamma$, the decrease in the surface tension of 5 mM phosphate solution caused by the minimum blocking concen-

TABLE 1

Surface activity-structure correlations of local anesthetics

Decrease in surface tension caused by the minimum blocking concentration (MBC) of local anesthetic without lecithin monolayer (column 2); decrease in surface tension caused by MBC of the local anesthetic at 100 Å²/molecule of lecithin monolayer (column 3); The MBC of the local anesthetic from reference (1) (column 4); the product of mole refraction R_0 and ionization potential I from reference (1) (column 5).

1	2 $-\Delta\gamma$ (dyne/cm)	3 $\Delta\pi$ (dyne/cm)	4 $-\log$ (MBC) (mM)	5 R_0I (cm²eV)
β-Naphthol	0.6	7.8	0.0	368
Thymol	0.0	5.6	0.52	412
Ephedrine	1.3	11.7	0.8	457
Procaine	1.0	12.8	1.67	543
Tetracaine	0.9	12.6	2.9	619
Phenyl-toloxamine	0.3	14.5	3.2	703
Quinine	1.8	14.7	3.6	750
Dibucaine	2.0	13.4	4.2	855

tration of the local anesthetic without the lecithin monolayer.

Figure 2 presents the plot of log $\Delta\pi$/MBC versus R_0I. It appears from this plot that the molecules presented in Table 1 follow the relation described by Eq. (4).

Equation (4) supports the previous model which resulted in Eq. (1). In other words, the partition function for the distribution of local anesthetic between the lecithin/water interface and the adjacent subsolution can be correlated with a molecular model which is based on the London interaction energy.

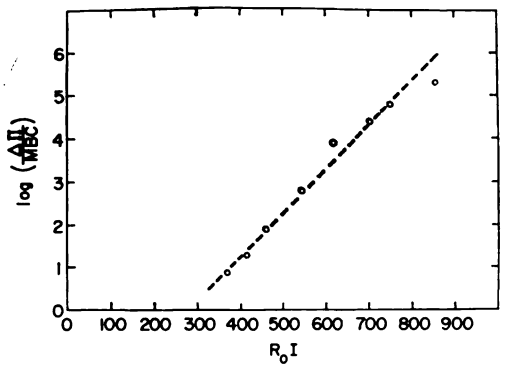

FIG. 2. *Plot of the logarithm of the ratio ($\Delta\pi$/MBC) (Table 1, columns 3 and 4) of 8 local anesthetics (Table 1, column 1) versus the product of the mole refraction R_0 and ionization potential I (Table 1, column 5)*

This suggests that the mode of interaction which causes the lowering of the surface tension involves the London interaction energy. There are many possible models consistent with this approach. Two primary examples are interaction between the nonpolar segments of the lecithin and anesthetic molecules to form a mixed monolayer (10), and interaction between the local anesthetic molecule and water molecules at the lecithin/water interface [e.g., clathrate formation (11, 12)] which in turn causes a decrease in the attraction between the polar groups of the lecithin molecule and the adjacent subsolution.

One partition function describes the distribution of the local anesthetic molecules in two different systems: (a) the distribution of local anesthetic molecules between the lecithin/water interface and the subsolution, and (b) the distribution between the site of local anesthetic activity and the extracellular solution. Namely, the results of this report lend support to the hypothesis that the site of local anesthetic activity is at the cell membrane and that interaction between nonpolar groups is of primary importance.

There is further evidence that ionic interaction is also involved in the blocking of nerve excitability. Many polyvalent ions such as Fe^{3+}, Al^{3+}, La^{3+}, Cr^{3+}, Co^{2+}, and Cd^{2+} reversibly block the action potential of nerve cells (13); and after the initial exposure of nerve to tertiary amine local anesthetics, lowering the pH of the external solution increases the effectiveness of these agents (14, 15). There are also reports describing ionic interaction between tertiary amine local anesthetics and phospholipids (13, 16).

A model incorporating both nonpolar and ionic interactions between local anesthetics and the site of activity has been suggested by Blaustein and Goldman (13). Inhibition of electrically induced changes in a phospholipid membrane system is caused by penetration of the lipid section by nonpolar agents (or segments) and by ionic interaction between local anesthetic and the phospholipid's polar head. A test of this model would be to examine the additivity of local anesthetic potency of cations such as Fe^{3+}, Al^{3+}, etc. and molecules such as toluene or β-naphthol.

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REFERENCES

1. D. Agin, L. Hersh and D. Holtzman, *Proc. Natl. Acad. Sci. U.S.* **53**, 952 (1965).
2. L. L. M. van Deenan, "Progress in the Chemistry of Fats and Other Lipids," Vol. 8, Part 1: "Phospholipids and Biomembranes" (R. T. Holman, ed.). Pergamon, London, 1965.

3. D. E. Green and J. F. Perdue, *Proc. Natl. Acad. Sci. U.S.* **55**, 1295 (1966).
4. J. F. Skou, *J. Pharm. Pharmacol.* **13**, 204 (1961).
5. J. F. Skou, *Acta Pharmacol. Toxicol.* **10**, 325 (1954).
6. N. L. Gershfeld, *J. Phys. Chem.* **66**, 1923 (1962).
7. G. L. Gaines, "Insoluble Monolayers at Liquid-Gas Interfaces," p. 45. Wiley (Interscience), New York, 1966.
8. J. F. Padday and D. R. Russell, *J. Colloid Sci.* **15**, 503 (1960).
9. D. O. Shah and J. H. Schulman, *J. Lipid Res.* **6**, 341 (1965).
10. J. H. Schulman and E. K. Rideal, *Proc. Roy. Soc.* **B122**, 29 (1937).
11. S. L. Miller, *Proc. Natl. Acad. Sci. U.S.* **47**, 1515 (1961).
12. L. Pauling, *Science* **134**, 15 (1961).
13. M. P. Blaustein and D. E. Goldman, *Science* **153**, 429 (1966).
14. J. M. Ritchie and P. Greengard, *J. Pharmacol. Exptl. Therap.* **133**, 241 (1961).
15. J. M. Ritchie, B. Ritchie, and P. Greengard, *J. Pharmacol. Exptl. Therap.* **150**, 152 (1965).
16. M. B. Feinstein, *J. Gen. Physiol.* **48**, 357 (1964).