Effects of Tetracaine and Hexobarbital on Artificial Phospholipid Membranes

A striking correlation has been demonstrated between the pharmacological activity of local anesthetics and their ability to interact with membrane lipids[1]. Studies with model systems[2-4] have shown that cationic local anesthetics (procaine and its derivates) interact mostly with negatively charged phospholipids, thus supporting the belief that the interaction between local anesthetics and membrane phospholipids is mainly electrostatic. However this type of interaction cannot explain the anesthetic properties of either neutral or anionic molecules, like alcohols[5] and barbiturates[6]. Evidence of the occurence of primary hydrophobic interactions, first suggested by SKOU[2] and strongly supported by SEEMAN[7], has recently been produced with NMR-analysis[8].

The purpose of this paper is to evaluate the effect of the surface charge on the interactions between artificial lipid membranes and two drugs with local anesthetic properties. We used tetracaine, a typical cationic local anesthetic and hexobarbital, a barbiturate, which dissociates as an anion. Both reduce and block the action potential in nerve fibres[9].

Materials and methods. Reagents (analytical reagent grade) were purchased from Merck (Darmstadt). Na-hexobarbital was purchased from Rhone-Poulenc (Paris) and Tetracaine-HCl was a gift of Hoechst (Frankfurt).

Phosphatidylethanolamine (PE) and phosphaitdylserine (PS) were separated by DEAE column chromatography from rat brain lipids. Their fatty acid composition was the same as reported by GALLI et al.[10], that is both phospholipids were found to be rich in fatty acids with one or more unsaturated bonds per molecule. The phospholipids were suspended in *n*-decane (20 mg/ml).

Table I. Resistance of PE and PS bilayer membranes in 100 m*M* KCl buffered solutions (intrinsic bilayer resistance, R_0) and maximum effect of the 2 anesthetics expressed by the ratio between the resistance produced by the drugs and R_0

	PE 6.4	PS 6.4	PS 8.4
$R_0(\Omega \cdot cm^2)$	4×10^5	2×10^6	2×10^5
$\frac{R\text{Hexob}}{R_0}$	40.5	28.5	3.7
$\frac{R\text{Tetrac}}{R_0}$	2.1	6.0	10.1

The values refer to a 0.1 m*M* drug concentration.

Table II. Oil/water partition coefficients (P) was determined according to JANSSON et al.[15].

	pK_a	P	
		pH 6.4	pH 8.4
Hexobarbital	8.24	0.13 (98.6)	0.05 (41.2)
Tetracaine	8.48	0.38 (0.8	2.55 (45.4)

The figures in parenthesis represent the calculated percentage of the neutral form.

[a] P was determined according to JANSSON et al.[15].

The bilayers were formed by the technique of MÜLLER and RUDIN[11] across a 1 mm² hole in a teflon partition separating 2 lucite chambers containing identical solutions of 100 m*M* KCl buffered at pH 6.4 with 5 m*M* *Tris*-maleate or at pH 8.4 with 5 m*M* *Tris*-HCl, kept at 23 °C (± 1). The anesthetics were always added into the 2 cell halves before forming the membranes. The electrical resistance was determined by applying a potential difference of 30 mV through a pair of Ag-AgCl electrodes and measuring the relative potential drop on the membrane with an electrometer.

Results and discussion. The resistance of our PE and PS untreated membranes is about 10 times lower than those reported in the literature[4,12]. This can be explained by the high degree of unsaturation of our phospholipid fractions. It is known that the resistance of films made with synthetic saturated lecithins is at least an order of magnitude higher than those of lecithins from natural sources[13]. Further, in our experiments, bilayers made with PE (unsaturation index 178[10]) show resistances lower than bilayers made with PS (unsaturation index 92[10]).

Tetracaine and hexobarbital at low concentrations (up to 1 m*M*) increase significantly the electrical resistance of a neutral bilayer (PE at pH 6.4) as reported in Table I. In fact, it has been demonstrated[14] that PE at pH 6.4 is a zwitterion with zero net charge. Since the two drugs produce the same modification of the bilayer permeability, it may be argued that they interact in the same way with PE membrane, probably through hydrophobic interaction between the non-polar groups of the anesthetics and the hydrocarbon chains of the phospholipids. CERBÓN[8] has clearly shown with NMR analysis that part of tetracaine can penetrate between the first carbon of a neutral bilayer.

In order to establish whether the increase of membrane resistance can be correlated with the liposolubility of the drugs, we determined the oil/water partition coefficient (P) of hexobarbital and tetracaine. At both pHs tetracaine has a higher P (Table II). Further, each drug was more soluble in the oil phase at the pH at which the non-ionized fraction (figures in parenthesis) prevails. The ability of the anesthetic to increase the resistance is not simply dependent on its liposolubility, since hexobarbital produces an increase larger than tetracaine, but has a smaller P. Other properties of the anesthetic

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molecule should be taken into account, such as bulkiness, rigidity and the ability to form hydrogen bonds with the phosphate oxygen of the phospholipids.

The presence of a net charge on the membrane surface does not qualitatively change the action of tetracaine and hexobarbital, but influences the increase of resistance produced by a given dose of anesthetic (PS bilayers, Table I). A -158 mV surface potential has been reported for the PS membranes at pH 7.2[12] and can be assumed to be present at the surface of PS bilayers in the pH range of our experiments[14]. This negative surface potential could modify the concentration of the anesthetic in a region near to the interface. Hexobarbital, which dissociates as an anion, should be diluted and tetracaine, which dissociates as a cation, should be concentrated with respect to the bulk aqueous solution. In fact, hexobarbital at 0.1 mM increase 40 times the resistance of the neutral PE at pH 6.4 and only 28 times the resistance of the strongly charged PS at pH 6.4. The opposite is valid for tetracaine: the more dense the surface charge, the more effective the drug.

It can be stated that the concentration of the drug in the membrane phase is determined by the partition coefficient and the interfacial concentration. The latter depends on the sign and density of the surface charge of the membrane and on the sign of the ionized form of the drugs in solution. Both factors are affected by the dissociation of the drug. However, the effect on the partition coefficient is the most important, as can be seen in the experiments with tetracaine on the PS membrane, where the pH change influences only the dissociation of the drug and not the surface charge of the membrane. Tetracaine is more active at pH 8.4, when the partition coefficient is higher and the ionized fraction is lower.

At concentrations near 1 mM, both drugs caused a large decrease of the resistance values (below those of the intrinsic bilayers) and finally (over 2–3 mM) the films tended to break into the solution. The drop in resistance was probably due to coarse rearrangement of the double layer structure, such as local micellizations.

The molecular mechanism by which the presence of the anesthetic in the bilayer produces an increase of resistance, is still not understood. We suggest that the insertion of relatively rigid structures like the anesthetic molecules in the bilayer can reduce the fluidity of the first carbons of the fatty acids. It is well known that cholesterol, which decreases the fluidity of artificial membranes[16], also reduces their permeability[17]. Local anesthetics, which increase bilayer rigidity, may thereby also increase the bilayer resistance.

Riassunto. Viene studiato l'effetto della carica elettrica sull'interazione tra farmaci ad azione anestetica locale e fosfolipidi di membrana. Viene messa in evidenza la natura principalmente idrofobica di questa interazione.

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Modification of Diuresis in the Rat by Chlorpropamide, Glibenclamide and Tolbutamide

Oral hypoglycaemic drugs are of increasing importance in the management of maturity onset diabetes. Some of these drugs have been implicated in disorders of water and electrolyte balance. For instance several workers have reported an antidiuretic action of chlorpropamide in water loaded normal subjects[1,2] water loaded subjects with diabetes mellitus[3] and in patients with diabetes insipidus[1,2,4–6]. Several mechanisms have been suggested. One that has received most support is that of EARLEY[7] who suggested that chlorpropamide potentiated the effect of ADH. Such a mechanism would be expected to result in hyponatraemia and serum hypoosmolarity, symptoms demonstrated by Weissman et al. in five diabetics treated with chlorpropamide. A clinical survey showed that about 4% of patients treated with chlorpropamide developed these symptoms. WEISSMAN[8] suggested that in these 4% the cessation of ADH secretion in response to developing hyponatraemia was incomplete so allowing ADH potentiation to continue and result in more pronounced symptoms.

Further mechanistic investigations using experimental animal preparations have shown, for instance, that using the toad bladder[9] chlorpropamide has no effect alone, potentiates small ADH levels but has no such effect on large doses of ADH. Similar results have been obtained in vivo using a strain of rats with genetic hypothalamic diabetes insipidus[10]. The same study showed no antidiuretic effect of chlorpropamide in normal rats perhaps because these animals compensated for the increased effectiveness of their ADH by reducing ADH output. In both untreated rats with diabetes insipidus and normal rats sodium excretion was significantly increased during chlorpropamide treatment which in the diabetic rats led to an increase in urine volume. This effect of chlorpropamide may be separate from any effect on ADH.

Glibenclamide in contrast to chlorpropamide has been reported to enhance diuresis in normal subjects, patients with diabetes mellitus[3] and those with diabetes insipidus[11]. MOSES et al.[3] have suggested that this effect is due to the

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