Adsorption Behaviour of Local Anaesthetics in Synthetic Lipid Membranes coated on a Quartz-crystal Microbalance and Correlations with their Anaesthetic Potencies[1]

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The partition coefficients in a lipid matrix of seven local anaesthetics in clinical use were obtained using quartz-crystal microbalance (QCM) coated with a synthetic lipid multibilayer film; the resonance frequency of the QCM changes linearly with the amount adsorbed by the lipid matrix. A good correlation was observed between the partition coefficients so determined and the intrinsic local anaesthetic potencies of the same compounds obtained from biological experiments: anaesthetics that induce strong local anaesthesia showed greatest partition into the lipid matrix. The partition coefficients depended on the phase transition of the lipid matrix on the QCM and the ambient pH in the aqueous phase. The uncharged form of amino-type local anaesthetics is strongly adsorbed by the lipid membrane and penetrates deeply into the lipid matrix, especially in the fluid liquid-crystalline state above the phase-transition temperature (T_c), compared with the cationic anaesthetic species present in acidic aqueous solutions.

Local anaesthetics are thought to interact with synaptic membranes site-specifically and to change the ion permeability of the membrane by blocking the sodium channels of cell membranes.[2] Since the chemical structures of local anaesthetics are not specific (cocaine, benzyl alcohol, amino-type aromatic compounds), the molecular mechanisms of the reception of local anaesthetics and the transaction process in synaptic membranes are not well understood. For example, whether blocking results from direct interaction of the anaesthetic with proteins[2] or from peturbation by the anaesthetic of the lipid matrix surrounding the channels[3,4] is still unclear. The first step may be the penetration of anaesthetic molecules into the lipid matrix, after which site-specific interaction with proteins in the membrane may occur.[5–8] The interaction of local anaesthetics with lipid membranes has been widely studied by various techniques including EPR,[9] X-ray diffraction[10] and ^{2}H NMR,[11] all of which suggest that the anaesthetics intercalate the lipid bilayer. However, there have been few studies to determine quantitatively partition coefficients of local anaesthetics in the lipid matrix and to compare these with the level of local anaesthesia.

We have recently reported partition processes and partition coefficients of odour,[12] bitter substances,[12] general anaesthetics[13] and antibiotics[13] in a lipid matrix by using a quartz-crystal microbalance (QCM) coated with a synthetic lipid membrane in an aqueous solution. QCMs are known to provide a very sensitive means of measuring mass at nanogram levels; the resonance frequency of the device changes sharply upon the deposition of a given mass on the electrode of the crystal plate.[14,15] Therefore, the lipid-membrane-coated QCM constitutes a useful device for detecting quantitatively partition processes of various bioactive compounds that adsorb into and penetrate site-specifically or non-site-specifically lipid membranes.[12,13,16–18]

In this paper we report partition coefficients of seven amine-type local anaesthetics (dibucaine, tetracaine, bupivacaine, lidocaine, prilocane, mepivacaine and procaine) in synthetic lipid matrices obtained using a lipid-coated QCM. A synthetic multibilayer film [dimethyldioctadecylammonium poly(styrene-4-sulphonate), $2C_{18}N^+2C_1/PSS^-$] and a synthetic and naturally occurring phospholipid (1,3-dihexadecylglycero-2-phosphoethanolamine, $2C_{16}PE$, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, DPPE) (Fig. 1) were used as the lipid matrix on a QCM. The experimental arrangement is shown in Fig. 2. A good linear correlation was found between the partition coefficients of local anaesthetics in a lipid matrix obtained by the QCM method and the intrinsic potencies of the anaesthetics obtained by *in vivo* experiments. Measured partition coefficients are affected by both the ambient pH and the phase transition of the lipid matrix. Structure–activity relations of local anaesthetics are also discussed.

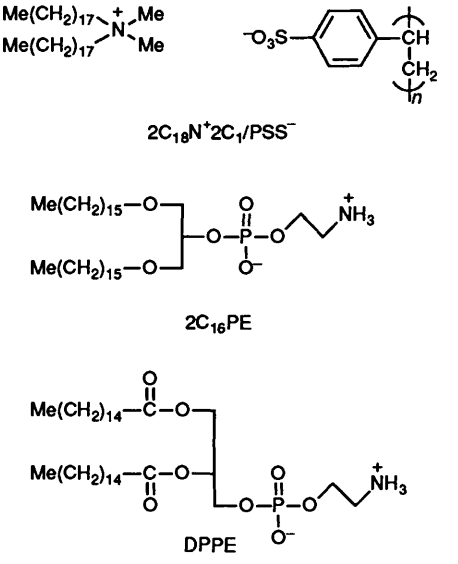

Fig. 1 Synthetic lipid membrane components

Experimental

Materials.—Preparations of the polyion-complex-type synthetic bilayer-forming amphiphile $2C_{18}N^+2C_1/PSS^-$ and the synthetic phospholipid $2C_{16}PE$ are reported elsewhere.[19,20] DPPE was commercially available from Sigma. Local anaesthetics dibucaine (DC), tetracaine (TC), bupivacaine (BC),

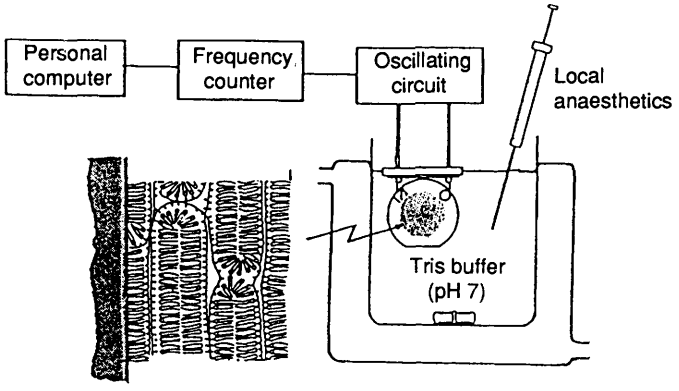

Fig. 2 Experimental set-up for frequency measurements of a lipid multibilayer film-coated quartz-crystal microbalance

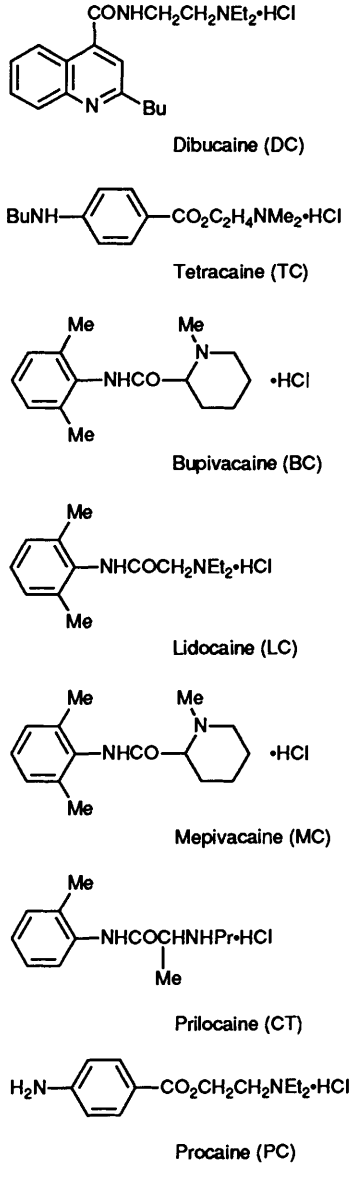

Fig. 3 Chemical structures of local anaesthetics

lidocaine (LC), prilocaine (CT), mepivacaine (MC) and procaine (PC) were used as their HCl salts (commercially available forms); their chemical structures are illustrated in Fig. 3.

Lipid-coated QCM.—A quartz-crystal microbalance (8 mm diameter, AT cut, 9 MHz) was connected to a home-made oscillator designed to drive quartz at its resonance frequency in aqueous solution.[12,13,16–18] The QCM was driven at 5 V DC, and the frequency of the vibrating quartz was measured by a frequency counter (Iwatsu Co., Japan, model SC7201) attached to a microcomputer system (NEC Co., Japan, model PC 9801) through a GP-IB board (see Fig. 2). Eqn. (1) was obtained for

$$\Delta F = \frac{-2F_0^2}{A(\rho_q \mu_q)^{\frac{1}{2}}} \Delta m \qquad (1)$$

the AT-cut shear mode QCM;[14,15] where ΔF is the measured frequency shift (Hz), F_0 is the parent frequency of the QCM (9×10^6 Hz), Δm is the mass change (g), A is the electrode area (0.20 cm^2), ρ_q is the density of quartz (2.65 g cm^{-3}) and μ_q is the shear modulus (2.95×10^{11} dyn cm^{-2}). Calibration of the QCM used in these experiments showed that a frequency change of 1 Hz corresponded to a mass increase of 1.05 ± 0.01 ng on the electrode [eqn. (2)].[12,13,16–18]

$$\Delta m = -(1.05 \pm 0.01) \times 10^{-9} \Delta F \qquad (2)$$

A chloroform solution (0.1 wt.%) of bilayer-forming amphiphiles ($2C_{18}N^+2C_1/PSS^-$, $2C_{16}PE$ or DPPE) was dropped and spread on both sides of the QCM electrodes, then dried in air. The cast film was aged in hot water at 60 °C for 1 h in order to obtain a well-orientated multibilayer structure.[12,13] X-Ray diffraction analyses showed that $2C_{18}N^+2C_1$ amphiphiles form extended lamellar structures of lipid bilayers (3.8 nm thick) parallel to the film plane (the QCM plate) in a polyion complex with poly(styrene-4-sulphonate) anions (PSS^-) as shown in Fig. 2.[19,20] The bilayer film showed a sharp endothermic peak at 45 °C with differential scanning calorimetry (DSC) in an aqueous solution, corresponding to the phase transition from the solid to the liquid-crystalline state.[19–21] When 10 ± 1 μg of the $2C_{18}N^+2C_1/PSS^-$ film was cast uniformly over the whole area of the electrodes (20 mm^2 × 2) on both sides of the QCM, the vibration frequency decreased by 10 500 ± 100 Hz both in air and in the aqueous solution, which was consistent with the mass deposited on the electrode in line with eqn. (2). Similar behaviour was observed when phospholipid ($2C_{16}PE$ and DPPE) multibilayer films were cast on the QCM. Although the Sauerbrey equation [eqn. (2)] was first applied to an oscillating QCM in air, the equation was confirmed to be applicable in the oscillating behaviour in liquid solutions: the frequency of the QCM decreases linearly depending on the mass deposited on the electrode in liquid solutions, although the fundamental resonance frequency decreases because of the liquid density and viscosity.[21]

The lipid-film coated QCM was soaked in a 0.1 mol dm^{-3} buffer solution (pH 3–12) and an aqueous solution (5–50 × 10^{-3} cm^3) of local anaesthetic was injected with stirring (see Fig. 2). It was confirmed from the frequency change that the $2C_{18}N^+2C_1/PSS^-$ multibilayer film was physically stable and did not peel from the QCM plate at the ng level even when subjected to the harsh conditions in aqueous solutions; it is chiefly for this reason that we chose the polyion complex film as a model of lipid membranes instead of the phospholipids (phospholipid cast films tend to detach partially from the plate in hot aqueous solution above their phase-transition temperatures, T_c).

Results and Discussion

Partition Coefficients of Local Anaesthetics.—Fig. 4 shows the frequency changes of a $2C_{18}N^+2C_1/PSS^-$ multibilayer film (10 μg, 0.5 μm thick) coated QCM responding to the stepwise addition of lidocaine hydrochloride into 10 cm^3 of aqueous solution (0.1 mol dm^{-3} Tris buffer, pH 7.0) at 45 °C. When 500

Table 1 Partition coefficients (P) in lipid films, anaesthetic intensities and toxicities of local anaesthetics

Local anaesthetics	log P in lipid films[a]			Intrinsic potency[b]	Toxicity[b]
	$2C_{18}N^+2C_1/PSS^-$	$2C_{16}PE$	DPPE		
Dibucaine	1.85	1.47	1.50	14	6.3
Tetracaine	1.66	1.52	1.55	9.5	4.3
Bupivacaine	1.59	1.32	1.23	5–10	5–10
Lidocaine	1.34	1.03	1.05	1	1
Prilocaine	1.31	1.03	0.99	0.65	0.77
Mepivacaine	1.26	0.74	0.75	0.55	0.81
Procaine	1.30	0.52	0.55	0.26	0.47

[a] Obtained at pH 7.0 and 45 °C according to eqn. (3) in the text. [b] Obtained from biological experiments on rabbits and normalized with values for lidocaine.

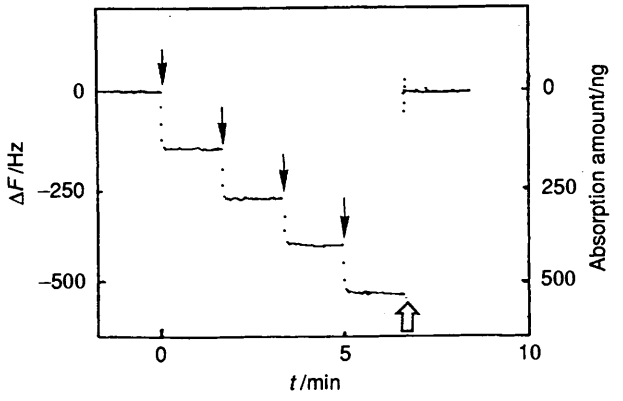

Fig. 4 Frequency changes of the $2C_{18}N^+2C_1/PSS^-$ film (10 μg) coated QCM responding to the stepwise addition of lidocaine (500 ppm, 1.9×10^{-4} mol dm^{-3} at each injection) into 10 cm^3 of 0.1 mol dm^{-3} aqueous Tris buffer solution (pH 7.0) at 45 °C. At the open arrow, the aqueous solution was changed for a fresh buffer solution.

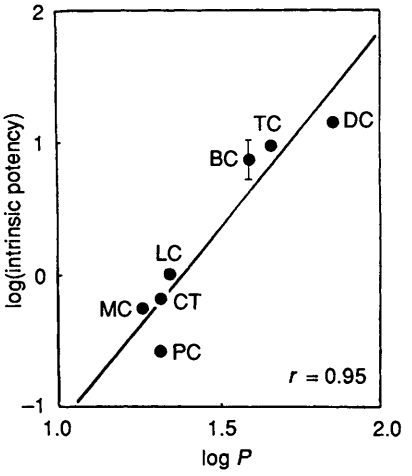

Fig. 5 Relation between partition coefficients (P) of local anaesthetics in the $2C_{18}N^+2C_1/PSS^-$ film on the QCM at 45 °C and the intrinsic potency of local anaesthesia for the same compounds. Abbreviations and chemical structures of anaesthetics are shown in Fig. 3.

ppm (1.9×10^{-4} mol dm^{-3}) of the anaesthetic was added at each injection, the frequency immediately decreased (ΔF = 135 ± 10 Hz) and reached the equilibrium value within 30 s, corresponding to an adsorption of 142 ± 10 ng in the QCM lipid matrix [from eqn. (2)]. The frequency reverted to the original value when the QCM was moved into a fresh buffer solution, indicating a desorption of lidocaine from the lipid matrix into the aqueous solution. This reversibility in adsorption–desorption behaviour could be repeated several times without damaging the membrane and was observed for other local anaesthetics. Partition coefficients (P) of local anaesthetics in the lipid matrix from the aqueous phase were obtained according to eqn. (3) where $[\text{anaesthetic}]_{\text{lipid}}$ and

$$P = \frac{[\text{anaesthetic}]_{\text{lipid}}}{[\text{anaesthetic}]_{\text{aq}}} \quad (3)$$

$[\text{anaesthetic}]_{\text{aq}}$ are the concentrations (mol dm^{-3}) of adsorbed anaesthetic in the lipid matrix (10 μg) and the aqueous solution, respectively.

Adsorption experiments were carried out on seven typical local anaesthetics—dibucaine, tetracaine, bupivacaine, lidocaine, mepivacaine, prilocaine and procaine—over a wide concentration range (1–1000 ppm, 10^{-5}–10^{-2} mol dm^{-3}). The amount adsorbed in the lipid film increased linearly with increasing concentration of these substances in the aqueous buffer solution at pH 7 (an example is shown in Fig. 4). Partition coefficients of local anaesthetics in the $2C_{18}N^+2C_1/PSS^-$, $2C_{16}PE$ and DPPE films were calculated according to eqn. (3) and are summarized in Table 1 along with the intrinsic potencies of local anaesthesia and toxicities of these compounds. The potency of local anaesthesia was referenced and obtained partly from skin irritant experiments on rabbits and was normalized by setting the intensity of lidocaine to unity.[4,22,23] The relative toxicity (LD_{50}) values, derived from intradermal injection studies on rabbits, were also normalized with the toxicity of lidocaine according to the literature.[4,22,23]

A good correlation (r = 0.95) was found between log P of various local anaesthetics in the $2C_{18}N^+2C_1/PSS^-$ film on the QCM and the logarithm of the intrinsic potency of local anaesthesia, as shown in Fig. 5. The plot of log P values *vs.* the toxicity of local anaesthetics also gave a good linear correlation (r = 0.91). The local anaesthetics giving the strongest physiological effects showed the highest partition towards the lipid matrix. Thus, the local anaesthetic activity or toxicity seems to be determined by the amount adsorbed in the lipid membrane. The similar good correlation between log P and log(intrinsic potency) was observed for $2C_{16}PE$ and DPPE phospholipid membranes (r = 0.94, not shown in the Figure). This means that the adsorption behaviour has very little dependence on the detailed structure of the hydrophilic part of the lipid matrix; the well-orientated dialkyl bilayer structure, on the other hand, is important for the adsorption step.

Partition coefficients of the seven local anaesthetics for protein matrices were also obtained, using a keratin-coated QCM at pH 7 and 45 °C. Keratin was chosen as a model of simple protein matrices because of its physical stability on the crystal plate at various conditions in aqueous solutions. The log P values were plotted *vs.* the logarithm of the intensity of local anaesthesia and are shown in Fig. 6. A significant correlation was not observed, which indicates that the intensity of a local anaesthetic depends on its partition to the lipid matrix but not to the hydrophobic part of the protein matrix. When the

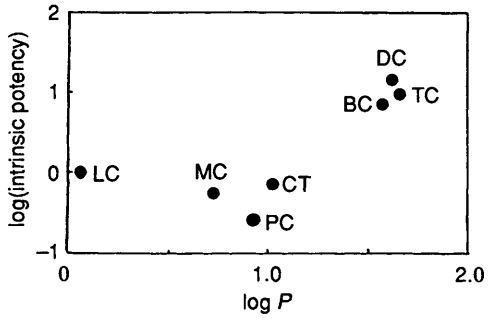

Fig. 6 Relation between log P of local anaesthetics in keratin proteins on the QCM and log(intrinsic potency) of local anaesthesia for the same compounds. Abbreviations and chemical structures of anaesthetics are shown in Fig. 3.

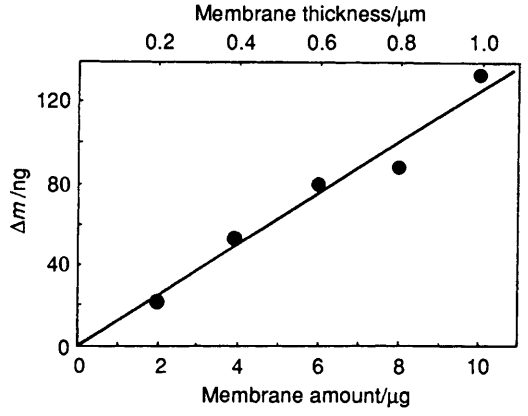

Fig. 7 Effect of the membrane thickness of the $2C_{18}N^+2C_1/PSS^-$ film on the QCM on the adsorption amount of lidocaine (500 ppm, 1.9×10^{-4} mol dm^{-3}) at 45 °C and pH 7.0

logarithm of the intrinsic potency of local anaesthesia was plotted *vs.* the partition coefficients between octanol and water phases for the same compounds, a significant correlation was not observed. This indicates that the activity of local anaesthesia is not determined simply by the hydrophobicity of the molecule. A plausible interpretation of these results is that the first step of local anaesthesia is the adsorption or penetration of anaesthetic molecules into the lipid bilayer matrix followed by an interaction with specific proteins in the membrane.[7,8,24,25]

Adsorption Mechanisms.—Fig. 7 shows the effect of the thickness of the $2C_{18}N^+2C_1/PSS^-$ multibilayer film on the QCM on the amount of lidocaine adsorbed, from which it can be seen that the amount adsorbed increases linearly with increasing membrane thickness. Similar adsorption behaviour was observed for the other local anaesthetics studied. Hence local anaesthetics must adsorb and penetrate deeply into the lipid multibilayer film.

Local anaesthetics are secondary or tertiary amines with pK_a values that lie between 8 and 9; in physiological solutions, therefore, they exist both as neutral and as cationic forms. Partition coefficients of tetracaine in the $2C_{18}N^+2C_1/PSS^-$ films were obtained at various ambient pH values (3–12) and the results are shown in Fig. 8. Partition coefficients increased discontinuously in alkaline solutions (above pH 8) compared with those in acidic solutions. Therefore, the uncharged form of tetracaine adsorbs and penetrates into the lipid matrix much more readily than the cationic species present in acidic solution.

The phase transition from the solid to the fluid liquid-crystalline state of the dialkyl chains is one of the fundamental properties of both natural and synthetic lipid bilayer mem-

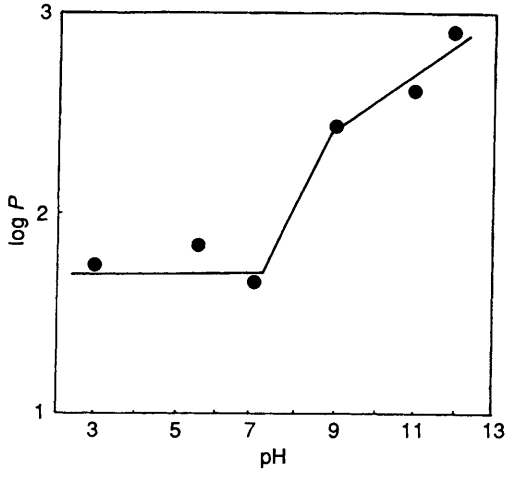

Fig. 8 Ambient pH dependence of partition coefficient of tetracaine in the $2C_{18}N^+2C_1/PSS^-$ film on the QCM at 45 °C

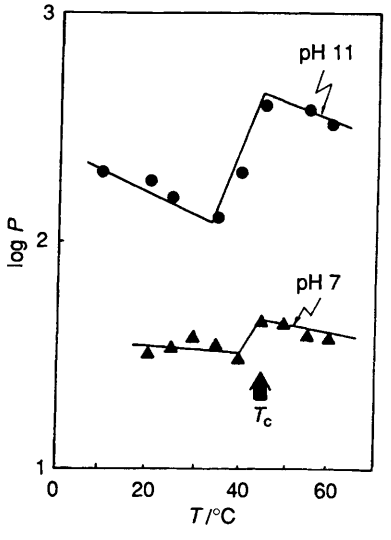

Fig. 9 Temperature dependence of partition coefficient of tetracaine in the $2C_{18}N^+2C_1/PSS^-$ film on the QCM at pH 7 and 11. The T_c value was obtained separately from the DSC measurements.

branes. Partition coefficients of tetracaine were measured on the QCM at various temperatures below and above the phase-transition temperature (T_c = 45 °C) of the $2C_{18}N^+2C_1/PSS^-$ multibilayer cast film both at pH 7 and 11; the results are shown in Fig. 9. The T_c value of the $2C_{18}N^+2C_1/PSS^-$ film was obtained separately by DSC in an aqueous solution (pH 7). Partition coefficients drastically increased at temperatures above 45 °C compared with those below T_c. Thus, local anaesthetics can adsorb and penetrate more easily into the disordered, fluid liquid-crystalline matrix (above T_c) compared with the solid matrix (below T_c). The log P values at pH 11 were larger than those at pH 7 throughout the whole temperature range studied, and the enhancement of the P values at T_c was larger at pH 11 than at pH 7. The neutral form of tetracaine can therefore penetrate easily into the fluid membrane above T_c compared with the cationic form. These results are consistent with the biological results: when local anaesthetics are applied to intact tissues, such as cornea or whole nerve trunks, they are usually more effective in alkaline solution (where the uncharged form is predominant) than in neutral solution (where the cationic form predominates).[24,25] This observation led to the belief that the uncharged form is the active form of the molecule at the nerve membrane.[26] Another proposal suggested that the cationic form is the active form, blocking a conduction *via* an

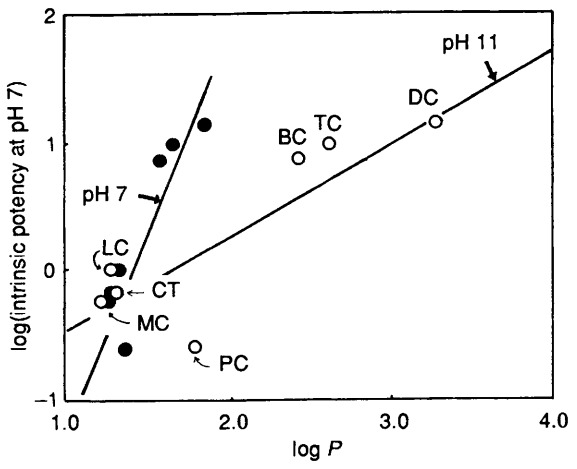

Fig. 10 Relations between log *P* values of local anaesthetics at pH 7 and 11 at 45 °C and log(intrinsic potency) of local anaesthesia. Abbreviations and chemical structures of anaesthetics are shown in Fig. 3.

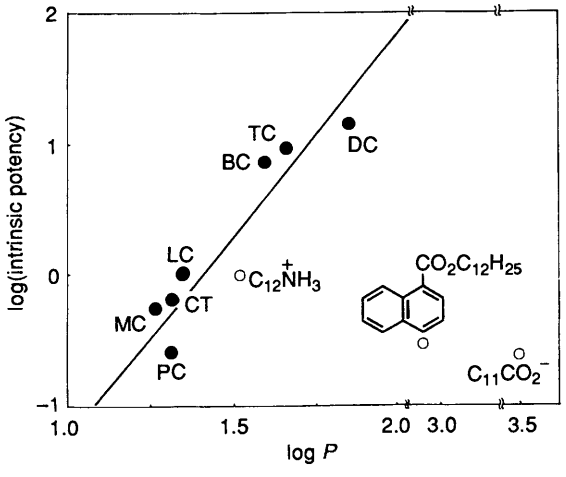

Fig. 11 Relation between log *P* values of seven anaesthetics and three general amphiphiles [sodium dodecanoate, dodecylammonium chloride and 1-(dodecyloxycarbonyl)naphthalene] in the $2C_{18}N^+2C_1/PSS^-$ film on the QCM and log(intrinsic potency) of local anaesthesia for the same compounds at 45 °C and pH 7.0. Abbreviations and chemical structures of anaesthetics are shown in Fig. 3.

electrostatic interaction with the nerve cell surface.[7] Our experiments indicate that the neutral form is more effective than the cationic form in adsorption and penetration into the lipid matrix, especially in the fluid liquid-crystalline state. The uncharged anaesthetic molecules would therefore penetrate into the tissue barrier more readily.

Fig. 10 shows correlations between the logarithm of the intrinsic potency of seven local anaesthetics and log *P* values at both pH 7 and 11. It is interesting to note that the log(intrinsic potency) values from biological experiments at neutral pH give a good linear correlation with the log *P* values at pH 11 as well as with those at pH 7. Partition coefficients of dibucaine, tetracaine, bupivacaine and procaine increase markedly with elevated pH. These compounds show strong local anaesthetic effects and, with the exception of bupivacaine, have two amino groups per molecule. Thus, the increased partition coefficients of these compounds in the uncharged form at pH 11 could largely be due to the neutralization of the two amino groups in the molecules.

In the mechanism of local anaesthesia, the first step seems to be adsorption and penetration of the anaesthetic molecule into the lipid matrix of the nerve membrane, followed by interaction or reception at a specific membrane protein. A specific chemical structure is therefore a prerequisite for local anaesthesia. This is different to the requirements for a general anaesthetic, in which the simple hydrophobicity (partition coefficients for octanol–water) of the molecule is the predominant activity factor.[27]

In order to study the specificity of the molecular structure for local anaesthesia, three general amphiphilic compounds—dodecylamine hydrochloride, dodecanoic acid and 1-(dodecyloxycarbonyl)naphthalene—which are not used clinically as local anaesthetics were chosen as additives. Partition coefficients to the $2C_{18}N^+2C_1/PSS^-$ film on the QCM and intrinsic potencies of local anaesthesia for these molecules were obtained from adsorption experiments and biological experiments, respectively. The results are plotted in Fig. 11 together with those for the seven local anaesthetics in common use. Dodecanoate anion and 1-(dodecyloxycarbonyl)naphthalene showed a large deviation from the linear correlation because of their very low intrinsic potency. In contrast, the dodecylammonium salt gave a point that lies nearly on the line. This means that dodecanoate anion and naphthalene ester can adsorb significantly into the lipid matrix but cannot interact with the specific protein in the biological membrane, giving rise to only weak local anaesthesia. The dodecylammonium salt, however, seems to penetrate into the lipid membrane and interact with the specific protein at levels similar to lidocaine and prilocaine. A plausible interpretation of these results is that the minimum requirement for the local anaesthetic activity is the amphiphilic nature of compounds with amino groups in the molecule, and that possession of aromatic hydrophobic groups is not an absolute requirement for the local anaesthesia.

Conclusions

Partition coefficients of local anaesthetics in a lipid matrix can be easily and quantitatively obtained by using the synthetic multibilayer film-coated QCM. A good correlation between the partition coefficients and the intrinsic potency of local anaesthesia obtained from biological experiments has been established. The synthetic-lipid-coated QCM will provide a new and simple sensor system for local anaesthetics, and should also be applicable for other bioactive compounds in which adsorption or penetration is predominant as the first step of the interaction with the biological cell membrane.

Acknowledgements

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