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Model for action of local anaesthetics with cytochrome oxidase

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In spite of the large number of studies carried out to elucidate the effect of local anaesthesia, the precise molecular mechanism by which local anaesthetics achieve this effect is not understood. The interaction of these compounds with cellular membranes plays an important part in their pharmacological action [1–3]. The understanding of these interactions is essential in the definition of an exact molecular mechanism of action [4–10]. We therefore decided to study the interactions between a series of eight local anaesthetics having different molecular structures with a model membrane system. This allowed the research of both the drug's affinity for membrane lipids and their functional effects on membrane proteins. Cytochrome oxidase seemed to be a good model membrane system on account of its structure [11, 12] and its well-defined functional properties [13–18].

Results and discussion

The drugs investigated were: procaine, lidocaine, prilocaine, carticaine, parethoxycaine, bupivacaine, pramocaine and quinisocaine.

To begin with [19] we measured the dissociation constant pK_a, the octanol–water partition coefficient as well as the anaesthetic activity of infiltration of the various compounds.

We then studied [20] the inhibitory effects of the same compounds on cytochrome oxidase enzymatic activity at the electron transfer level from cytochrome *c* to molecular oxygen. The "local anaesthetic affinity" for the enzyme was estimated from the reciprocal of the inhibition constant K_i determined for each compound.

Finally, direct association of local anaesthetics with cytochrome oxidase was investigated [21] from a study of the binding of the anaesthetics molecules to the enzyme. Association of anaesthetics to quinacrine bound cytochrome oxidase was studied quantitatively from the rate of fluorescence quenching, K, caused by the presence of local anaesthetics. K is the quenching constant measured from the Stern Volmer plots.

In Table 1 are reported all the parameters determined from the precedent studies; the various correlations established between these parameters are illustrated by the relationships presented in Table 2. The two different ways of studying local anaesthetic interactions with cytochrome oxidase allowed us to measure two rates of interaction, K_i^{-1} and K, which correlate quantitatively, as can be seen in equation (2) (Table 2). The following comments suggest that the results are also closely related qualitatively in several ways.

The site of local anaesthetics action appears to deal with cytochrome oxidase associated phospholipids. Indeed, local anaesthetics inhibit cytochrome oxidase activity in the low affinity interaction of cytochrome *c* which is thought [22] to account for the binding of cytochrome c to the enzyme lipidic environment. The inhibition process can be explained by a competition between local anaesthetics and cytochrome *c* to bind to the enzyme associated phospholipids and, the affinity of drugs for enzyme, K_i^{-1}, could describe the amount of anaesthetic interaction with phospholipids involved in the enzymatic activity. Also, local anaesthetics decrease the fluorescence of quinacrine bound to a region of low polarity in the cytochrome oxidase complex, probably with phospholipids of the enzymatic complex, and so the quenching constant K corresponds to

Table 1. Variation of the rate of interaction of local anaesthetics with cytochrome oxidase as a function of anaesthetic activity and physicochemical parameters

	Physicochemical parameters				Inhibition of cytochrome oxidase activity:		Quenching of cytochrome oxidase bound quinacrine: Stern–Volmer constant $K(M^{-1})$
Anaesthetics	Dissociation constant pK_a	Octanol–water partition coefficient log P	Ionization state [I]/[N]* pH 7	Anaesthetic activity of infiltration (A.A.I.)	Inhibition type	Affinity constant K_i^{-1} (M^{-1})	
Procaine	9.05	1.98	112.2	1	Mixed-type	78	1.9
Lidocaine	7.92	1.90	8.3	4.2	Mixed-type	92.6	6.8
Prilocaine	7.6	1.36	4	4.5	Non-competitive	53.8	6.5
Carticaine	7.45	1.26	2.8	6.3	Competitive	166.7	8.1
Parethoxycaine	8.08	2.22	12	7	Competitive	142.9	53.6
Bupivacaine	7.27	1.60	1.9	10.1	Competitive	188.7	15.2
Pramocaine	6.24	2.09	0.17	17.7	Competitive	555.6	984
Quinisocaine	6.30	2.50	0.2	55.8	Competitive	1408.5	5309

* [I]/[N]: relative amounts of ionized and neutral form of anaesthetics.

Table 2. Correlations between structure, activity and rates of interactions with cytochrome oxidase of a series of eight local anaesthetics

Structure–activity relationship:	Correlation between the rates of interaction of local anaesthetics with cytochrome oxidase represented by K_i^{-1} and K:	Role played by physicochemical parameters of anaesthetics molecules at anaesthetic–enzyme interactions:	Correlations between the rates of anaesthetic–enzyme interactions and pharmacological activities of drugs:
$\log AAI = 0.27 \log P - 0.48\ pK_a + 3.95$ (1) $N = 8$, $R^2 = 0.91$ $F = 24.6$, $F_{(2,5)1\%} = 13.3$ the correlation is significant at 1%.	$\log K = 2.4 \log K_i^{-1} - 3.88$ (2) $N = 8$, $r = 0.94$ $t = 6.76$, $t_{(6)0.005} = 3.707$ the correlation is significant at 0.5%.	$\log K_i^{-1} = 0.51 \log P - 0.36\ pK_a + 4.01$ (3) $N = 8$, $R^2 = 0.875$ $F = 17.45$, $F_{(2,5)1\%} = 13.3$ $\log K = 1.49 \log P - 0.93\ pK_a + 5.73$ (4) $N = 8$, $R^2 = 0.97$ $F = 71.3$, $F_{(2,5)1\%} = 13.3$ the correlations are significant at 1%.	$\log K_i^{-1} = 0.82 \log AAI + 1.56$ (5) $N = 8$, $r = 0.89$ $t = 4.72$, $t_{(6)0.005} = 3.707$ $\log K = 2.151 \log AAI - 0.306$ (6) $N = 8$, $r = 0.91$ $t = 5.48$, $t_{(6)0.005} = 3.707$ the correlations are significant at 0.5%.

drug interaction with the fluorophore phospholipid environment [23].

The examination of the various processes of interaction shows the existence of electrostatic interactions between anaesthetic molecules and phospholipids. The competitive type of inhibition, observed for most compounds and for the more potent inhibitors (cf. Table 1) suggests the existence of such interactions since the cytochrome *c* binding involves electrostatic interactions [15] particularly with negatively charged phospholipids [24, 25]. A similar remark can be made from the quenching phenomena observed for all the compounds (for the most part in a high ionization state), considering that the quenching process is the result of a contact between the fluorophore and the quencher molecule. These results agree with those of Surewicz [26] and Lee [27, 28] which indicate that both neutral and charged molecules can incorporate into lipidic membranes.

The existence of hydrophobic interactions is indicated by the correlations presented in equations (3) and (4) (Table 2) between the anaesthetic–phospholipid interaction rates and octanol–water partition coefficients of drugs. Indeed, the more lipophilic compounds have the greatest effectiveness of interaction (cf. Table 1). Additionally, the negative sign of the pK_a coefficient shows that the neutral form of the molecule is the most potent form. A similar observation has been made by Sikaris [29] for local anaesthetic interactions with phospholipid bilayers.

The establishment of electrostatic and hydrophobic interactions between anaesthetics and cytochrome oxidase associated phospholipids is in good agreement with the results of Boulanger [30] on local anaesthetics binding to lipidic membranes. These suggest that there are at least two binding sites for each of the charged and uncharged forms of the drugs in membranes.

Because of these results we suggest a model for the interaction of local anaesthetics with cytochrome oxidase based on the ability of molecules to penetrate the lipid layer surrounding the oxidase protein, with phospholipid-anaesthetic interactions of electrostatic and hydrophobic types. This model considers the access of molecules to a site on the enzyme boundary phospholipids [11, 12] which play a primordial role in enzymatic activity such as cardiolipin [13, 14, 17]. For this reason, it can explain the great capacity of the most lipophilic and the least ionized molecules (cf. Table 1) to inhibit enzymatic activity. In the same way the quenching phenomena can be explained by the ability of local anaesthetic molecules to reach the binding site of quinacrine on oxidase associated phospholipids. This model can also account for the low rates of interaction observed for the most ionized molecules. Indeed numerous electrostatic interactions could immobilize molecules at the surface of the annular lipid bilayer and so keep them from reaching an active site for inhibition or quenching. Strongly ionized molecules could therefore bind to a peripheral site of the annular lipid phase surrounding protein and thereby modulate the enzyme activity, when weakly ionized and lipophilic molecules could compete with cytochrome *c* [13] or quinacrine for binding at the active site located deeply in the lipid phase.

Interestingly, it can be noted that the interaction process between local anaesthetics and cytochrome oxidase is comparable, from various points of view, to membrane–anaesthetic interactions involved in their pharmacological action.

The appearance of local anaesthetic interaction with oxidase associated phospholipids agrees with the results of various studies on local anaesthetic interactions with both biological and model membranes [31–36] which tend to prove drug penetration of the lipid bilayer.

The existence of electrostatic interactions can be compared with both the observations of Papahadjopoulos [32] which indicate that local anaesthetic action on biological membranes involves specific interactions with acidic phospholipids, and the model of local anaesthetic interaction with the different molecular components of cellular membranes proposed by Wang [9]. This latter includes electrostatic interactions of cationic anaesthetics with both membrane proteins and anionic sites of phospholipids.

The part played by electrostatic and hydrophobic interactions of the charged and uncharged fragments in anaesthetic action mechanisms at cytochrome oxidase is compatible with the assumed molecular orientation of local anaesthetics in membrane. This orientation suggests that the polar head of the amine group is located at the polar–non polar membrane interface [36], when the hydrophobic part is embedded in the hydrocarbon region of lipid bilayer [33, 37–41].

We also defined a model of anaesthetic action at cytochrome oxidase showing striking similarities with the molecular mechanism of action of these drugs at the nerve cell membrane proposed by Richards [42] and Wang [43] who suggest that interactions of anaesthetics with lipids affect the functionally required lipid–protein interactions in the membrane bilayer.

The significant correlations observed between physicochemical properties and the anaesthetic activity of drugs on one hand and their rates of interaction with oxidase [cf. equations (1), (3) and (4)] on the other, indicate that the liposolubility and ionization state of compounds play the same role in each case. Indeed the greatest interactions and activities are observed for the most lipophilic and least ionized compounds (cf. Table 1). This similarity in structure activity relations agrees with the results of Hille [44, 45] which indicate that local anaesthetics externally applied to the membrane have access to their action sites only through hydrophobic pathways, which concern essentially the neutral form of molecules [2, 46–52].

Study of local anaesthetic interactions with cytochrome oxidase taken as a model membrane system provided a number of useful comparisons to the action of the same compounds on nerve membranes. The striking similarities of drug–membrane interactions in each case suggest that the results of our investigations could perhaps be extrapolated to the pharmacological mechanism of action of local anaesthetics at the molecular level. Particularly, the correlation between the rate of interactions of compounds with cytochrome oxidase associated phospholipids and their anaesthetic activities [cf. equations (5) and (6), Table 2] can be added to similar results to emphasize both the importance of local anaesthetic interactions with lipids at their interactions with nerve membranes [53] and the part played by phospholipids in excitation phenomena [32]. Finally, the action mechanism of local anaesthetics on cytochrome oxidase [54], which is principally based on drug interactions with oxidase associated phospholipids involved in the enzymatic activity, gives further support to the hypothesis of a molecular action mechanism of local anaesthetics based on drug interactions with membrane proteins at the site of the annular lipids–protein interactions which are required for the maintainance of the protein's biological function.

In summary, a model for action of local anaesthetics at cytochrome oxidase is defined which accounts for the various phenomena observed. This model is based on the ability of molecules to penetrate the lipid layer surrounding oxidase protein and fits closely to probable molecular mechanisms of action of local anaesthetics on nerve membrane.

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Hepatic drug-metabolizing enzymes in lung tumor-bearing rats

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Drug metabolism, which is subject to modification by many physiological factors [1], may also be altered in many pathological situations. A number of reports indicate that hepatic drug metabolism is impaired in tumor-bearing animals [2–5]. This impairment has been shown to be associated with decreased levels of hepatic cytochrome P-450 and b_5, and NADPH cytochrome *c* reductase [5–7]. It has also been reported that hepatic microsomal enzymes obtained from tumor-bearing animals metabolize several drugs at a lower rate than liver microsomal enzymes prepared from normal rats [3, 8]. A relevant study in this line shows increased serum antipyrine half-life in patients with hepatic malignancies [9]. These changes have been attributed to the defective metabolizing capacity of the uninvolved tissue of