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THE ACTION OF DRUGS ON MEMBRANES

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As far as membrane action is concerned, drugs can be classified as either hydrophilic or hydrophobic. Hydrophilic drugs are likely to bind to sites on membrane proteins much like those on normal water-soluble proteins, and they will show relatively little non-specific binding to the membrane. Most drugs having an effect on membrane proteins, however, are hydrophobic and can be expected to show extensive binding to the membrane.

A number of modes of interaction are possible for the hydrophobic drugs. Firstly, activity could follow indirectly from binding to the lipid component of the membrane. Two ways can be envisaged for linking such binding to an effect on protein function. Binding to the lipid component of the membrane could affect the fluidity of the lipid (either the bulk lipid or the annular lipid around the membrane proteins) and it is known that at least some membrane proteins are sensitive to the fluidity of the surrounding lipid (3). Alternatively, if the drug is charged, then the binding of such a drug will alter the charge on the membrane. Since most enzyme substrates, transmitters etc. are also charged, their concentrations close to the membrane surface will be altered with consequent alterations in enzyme activity, receptor activation etc. Further possibil-

ities arise from direct interaction of drugs with receptors in the membrane. For hydrophobic drugs a likely binding site is at the lipid-protein interface. Such binding could produce directly a conformation change in the protein. Alternatively, in binding to the protein, the drug could displace a lipid that was originally next to the protein, and it could be the displacement of this lipid that leads to the observed effect. If the receptor is a multi-subunit protein, as is the acetylcholine receptor for example, other possible binding sites lie at the interfaces between the subunits. A final class of binding site lies 'within' the protein. Henderson et al (2) have proposed a structure for the membrane protein bacteriorhodopsin consisting of five loops across the membrane. A drug could possibly bind between these loops resulting in major conformational changes.

A priori one would expect direct binding to membrane proteins to show more structural specificity than is shown by indirect effects on proteins mediated via binding to lipids. Thus many theories of local anaesthetic action have postulated lipids as the principal binding site (5).

The most direct way to study these possible types of interaction is by the use of spectroscopic techniques: our studies have mostly used fluorescence spectroscopy. All forms of spectroscopy are generally most powerful when applied to simplified and defined systems. Unfortunately, not many membrane proteins have yet been purified in sufficient quantity, but two that have are the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ from sarcoplasmic reticulum and the acetylcholine receptor from the electric organ of electric fish.

We will firstly look at drug interactions with phospholipid bilayers. This is particularly significant since it is known that the phospholipid composition of many organs, including brain, change during growth and development (1). We can detect effects of drugs on membrane fluidity using the fluorescence polarization of the probe diphenylhexatriene (DPH). We have found that although some local anaesthetics increase membrane fluidity and some decrease it, all effects are too small to be of any obvious physiological significance. The increase in fluidity caused by the concentration of octanol that causes 50% nerve block, for example, is equivalent to increasing the temperature of the system by 0.2°C . If fluidity is to be important, therefore, it has to be amplified in some way, and a possible

mechanism for this has been suggested (5).

Of more likely importance is the change in surface potential of the membrane that follows from binding of charged drugs to lipids. For charged drugs, binding cannot be described by a simple partition coefficient, but rather in terms of binding constants. Such binding constants can be derived from measurements of the effects of drugs on phase-transition temperatures of lipids. Binding constants are different for different phospholipids (4). Using these binding constants, it is possible to calculate the amount of drug bound to a membrane under any particular set of conditions, and then to calculate the charge on the membrane. These calculations agree well with the membrane charge measured by observation of movement of the membrane in an electric field. With positively charged drugs like the antihistamines, for example, it is possible to generate surface potentials of 50 to 60 mV in this way. Such potentials will have a marked effect on the activity of a protein such as the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ in the membrane, since the concentrations of the substrate Ca^{2+} close to the membrane surface will be much reduced as a result of charge-charge repulsion.

It is more difficult to study drug binding to the protein component of the membrane. Membrane proteins cannot be purified in a stable state free of lipids, so that it is always necessary to study lipid-protein systems. The problem is then to distinguish drug binding to the lipid component of the membrane from drug binding to the protein component. We have developed two techniques whereby this can be achieved.

In the first we make use of fluorescence probes which will report back to us the nature of their environment. One class of such probes are drug analogues containing the dansyl group. As shown in Fig. 1, the fluorescence emission spectrum for a dansyl-containing compound when bound to the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ shows two components, one of which can be associated with lipid-bound drug and the other with protein-bound drug. The relative intensities of the two components can be used to derive binding constants for the two types of site. Interestingly, with the acetylcholine receptor, binding to the lipid component of the membrane is much reduced, and binding to the receptor appears to be relatively specific (6).

The second method makes use of the fluorescence quenching

of the tryptophan residues on membrane proteins caused by bromine-containing phospholipids. Addition of normal phospholipids to the membrane displaces the brominated lipid, and is detected as an increase in fluorescence (Fig. 2). Similarly, addition of a drug than can bind at the lipid-protein interface will cause an increase in fluorescence, from which it is possible to derive data for the binding of that drug.

Whereas drug interactions with the acetylcholine receptor appear relatively specific (6) this is not so for the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$. However, the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ can show some specificity. Thus we have confirmed the experiments of Warren et al. (7) that suggest that cholesterol is excluded from the annulus around the $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ since it has no effect on the activity of the ATPase when a full complement of annular lipids is present. On the other hand, we have observed that negatively charged cholesterol analogues such as cholesterol sulphate strongly inhibit the activity of the ATPase.

The factors determining the effect of a drug on a membrane protein are clearly very subtle. Using the fluorescence techniques outlined above we are in a position to 'map' the binding sites for hydrophobic drugs on membrane proteins, and to separate out some of the effects that could follow from drug binding to the lipid component of the membrane.

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Figures

Fig. 1 Fluorescence emission spectrum of dansyl undecanoic acid bound to the $(Ca^{2+}-Mg^{2+})$ -ATPase, X experimental points. Solid line-best least squares fit to the data. Dotted lines, the protein and lipid components from the least squares fit.

Fig. 2 The effect of an increasing percentage of brominated-dioleoylphosphatidyl choline on the fluorescence of $(Ca^{2+}-Mg^{2+})$ -ATPase, and the effect of hexadecyl trimethylammonium bromide on the fluorescence intensity of $(Ca^{2+}-Mg^{2+})$ -ATPase in the presence of 100% brominated-dioleoyl phosphatidylcholine.

Fig 1

Fig 2

TOPOLOGY AND BARRIER FUNCTION OF MEMBRANES IN THE LUNG

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SUMMARY

The alveolo-capillary air-blood barrier of the lung is made of two thin cell layers, epithelium and endothelium, that limit the flow of solutes and fluid from blood plasma into the narrow interstitial space and into the very thin alveolar fluid lining associated with surfactant. The conditions for maintaining a thin dry air-blood barrier are discussed. The effect of barrier thickness on gas exchange is discussed by means of the changes in barrier structure that lead to respiratory failure in the respiratory distress syndrome.

INTRODUCTION

The lung and the brain are the two organs where one finds the highest concentration of cell membranes per unit volume of tissue. In the lung these membranes are used to establish an intense contact between air and blood over an area of about 140 m² in the adult, an area which measures some 60 m² even in a 10-year old child. Membranes are used to build a barrier sufficiently thin for efficient gas exchange between air and blood.