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# THE MOLECULAR ORGANISATION OF BIMOLECULAR LIPID MEMBRANES

## THE EFFECT OF KCl ON THE LOCATION OF INDOLEACETIC ACID IN THE MEMBRANE

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## Summary

The effect of indoleacetic acid on the individual dielectric and conductance parameters of the polar-head, hydrocarbon and unstirred surface-layer regions of bimolecular lipid membranes was studied using low frequency (0.1—100 Hz) impedance dispersion measurements. It was found that the effect of $10^{-6}$ M indoleacetic acid on these parameters was dependent on the concentration of KCl in the external solution. Thus in 1 M KCl the conductance of the hydrocarbon region increased from 2 to 26 $\mu\Omega^{-1}/cm^2$ in the presence of indoleacetic acid while the surface layer conductance was largely unaltered. In 1 mM KCl, however, the hydrocarbon region conductance was virtually unchanged by indoleacetic acid whereas the surface layer conductance decreased from 3000 $\mu\Omega^{-1}/cm^2$ to 1100 $\mu\Omega^{-1}/cm^2$. The average value of the polar-head capacitance, which was not dependent on the KCl concentration decreased by a factor of 2 in the presence of $10^{-6}$ M indoleacetic acid. A similar reduction in the conductance of this region was also observed. The hydrocarbon capacitance was insensitive to the presence of indoleacetic acid at all KCl concentrations. It is concluded that at low KCl concentrations indoleacetic acid is located only on the surface and in the polar-head regions of the membrane while at high concentrations of KCl indoleacetic acid is absorbed into the hydrocarbon region, leaving the surface largely in its unmodified state. These results correlate with the known salt dependence of the action of the hormone in plant cells.

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## Introduction

There is a good deal of evidence that the plant growth hormone indoleacetic acid affects ion transport in membranes through stimulation of active transport or carrier mechanisms (e.g. see refs. 1—6). It has also recently been shown [7,8] that indoleacetic acid at very low concentrations ($10^{-5}$ —$10^{-8}$ M) exerts a direct influence on some pressure dependent electrical parameters of the membranes of *Valonia utricularis*. In order to shed some light on this subject it would be useful to know more about the localisation of absorbed indoleacetic acid molecules within the membrane. Information on the molecular organisation of membranes can be obtained from the very low frequency (0.1—100 Hz) dispersion of the electrical impedance [9]. From such measurements it was possible to characterise the separate dielectric and conduction parameters of the polar and hydrocarbon regions in phosphatidylcholine bimolecular lipid membranes [9] and to determine variations in these parameters when the membrane was modified by substances taken up from the external solution [10]. In this communication we report results of such experiments on the effect of the external concentration of KCl, on the location or penetration of indoleacetic acid molecules into artificial, phosphatidylcholine, bimolecular lipid membranes.

## Materials and Methods

The membranes were made by the common technique of painting a film of *n*-tetradecane containing egg-phosphatidylcholine over a hole (1.2 mm in diameter) in a polycarbonate septum which divided a plexiglass cell into two compartments. The cell was filled with a 1, 10, 100 or 1000 mM KCl solution containing either zero or $10^{-6}$ M indoleacetic acid. The pH was adjusted to 5.5. The p$K$ of indoleacetic acid is ≈4.8 so at pH 5.5 the molecule should be fully dissociated. Some spot measurements were done at pH 7 to confirm the non-dependence on pH. Membranes would not form readily below pH 4.8.

The overall admittance $Y$ of the membranes (for an equivalent circuit see Fig. 6) at an angular frequency $\omega$ is given by

$$Y = (Y_H^{-1} + Y_P^{-1} + G_\ell^{-1})^{-1}$$

were $Y = G + j\omega C$;   $Y_H = G_H + j\omega C_H$;   $Y_P = G_P + j\omega C_P$.   (Eqns. 1).

Here $G$ and $C$ are the overall frequency dependent conductance and capacitance respectively, $G_H$ and $C_H$ are the hydrocarbon region conductance and capacitance respectively, $G_P$ and $C_P$ are the polar head region conductance and capacitance respectively, $G_\ell$ is the conductance of the modified surface layer * and $j = \sqrt{-1}$. From measurements of $C$ and $G$ as a function of frequency it is possible to determine the values of $C_H$, $G_H$, $C_P$, $G_P$ and $G_\ell$ (for full details see [10,11]).

* The reactive impedance of such layers (i.e. that due to the diffuse ionic double layer [12]) is small in comparison with the experimentally measured conductance $G_\ell$ at frequencies below ≈100 Hz. For simplicity therefore, the capacitance of this layer is not specifically included.

## Results

*(a) Determination of the polar-head, hydrocarbon and surface-layer parameters*

Examples of the capacitance and conductance of a phosphatidylcholine bimolecular lipid membrane made in 1 mM KCl without indoleacetic acid and with $10^{-6}$ M indoleacetic acid, as a function of frequency, are shown in Fig. 1.

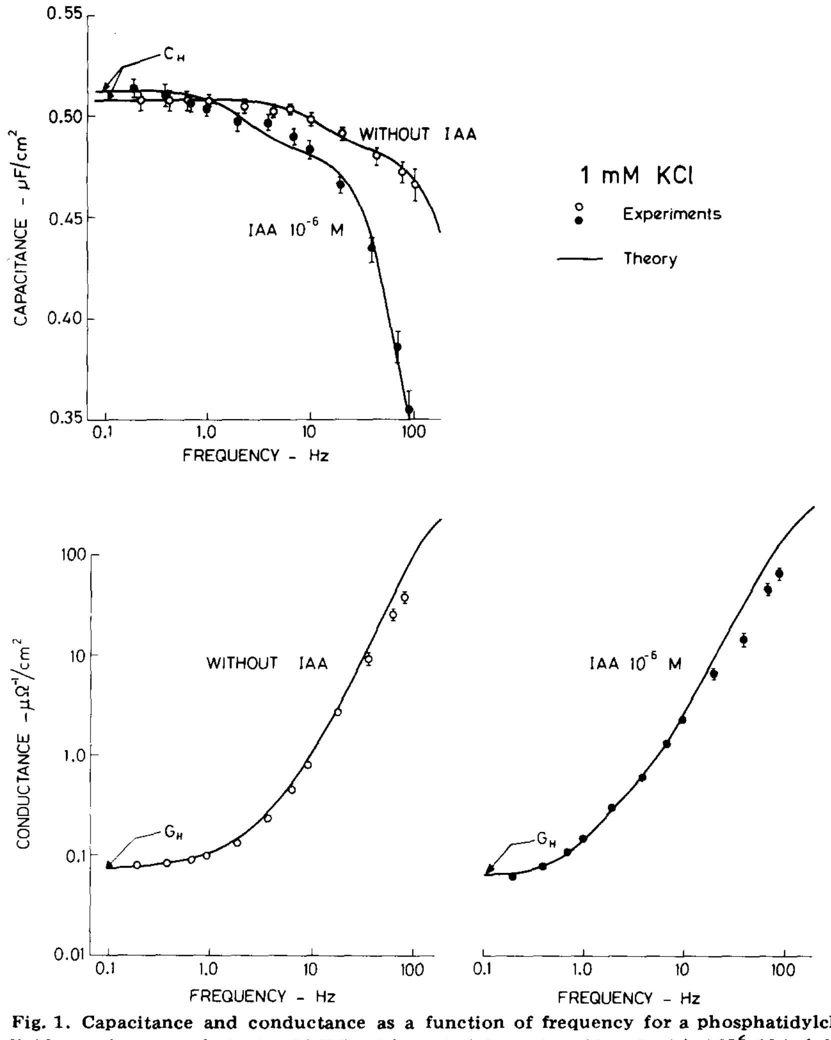


Fig. 1. Capacitance and conductance as a function of frequency for a phosphatidylcholine bimolecular lipid membrane made in 1 mM KCl without indoleacetic acid and with $10^{-6}$ M indoleacetic acid in the external solution. For clarity the results for the conductance with and without indoleacetic acid are shown separately since the plots overlap. The points shown are the average, at each frequency, of five runs on these membranes. The vertical bars indicate the standard errors (for the conductance data these are difficult to discern on these plots). The full curves are the theoretical dispersions predicted by Eqns. 1 with the values of $C_H$ and $G_H$ indicated on the diagram. The other parameters had the following values; without indoleacetic acid, $C_P$ = 22 $\mu$F/cm², $G_P$ = 650 $\mu\Omega^{-1}$/cm², $G_Q$ = 3000 $\mu\Omega^{-1}$/cm². Correlation coefficient between theory and data was 0.95 for $C$ and 0.85 for $G$; with $10^{-6}$ M indoleacetic acid, $C_P$ = 16 $\mu$F/cm², $G_P$ = 300 $\mu\Omega^{-1}$/cm², $G_Q$ = 900 $\mu\Omega^{-1}$/cm². The correlation coefficients were 0.90 for $C$ and 0.75 for $G$. IAA, indoleacetic acid.

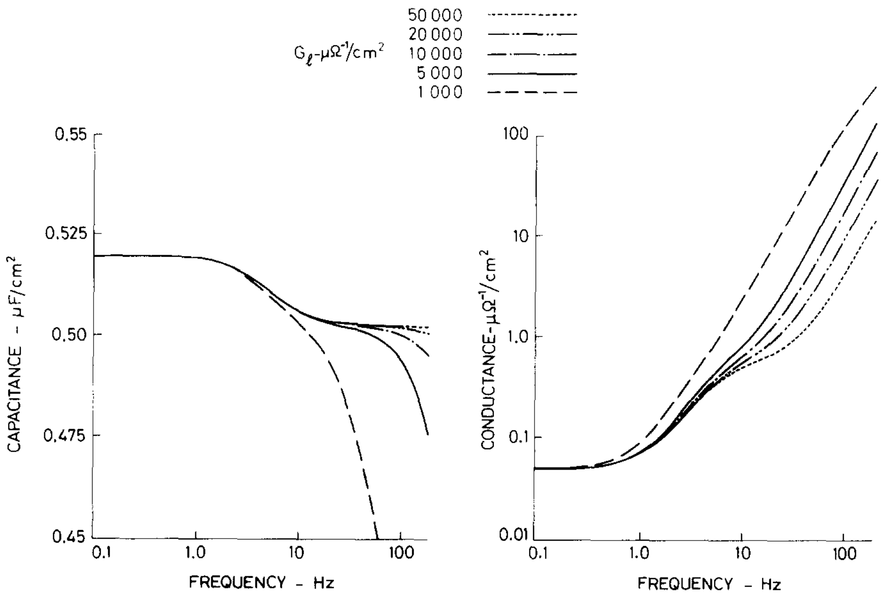


Fig. 2. The effect of the surface layer conductance element, $G_\ell$, in the unstirred solution layer, on the dispersion characteristics. As $G_\ell$ decreases, the dispersion due to the presence of this element shifts towards lower frequencies and when $G_\ell = 1000\ \mu\Omega^{-1}/\text{cm}^2$ merges with the dispersion due to the presence of the polar-head region. Calculations show that the magnitude of the hydrocarbon region conductance, $G_H$, does not significantly alter the effect of $G_\ell$. The values of the other parameters used in the calculations (typical of a bimolecular lipid membrane in 1 mM KCl in the absence of indoleacetic acid) were: $C_H = 0.52\ \mu F/\text{cm}^2$, $G_H = 0.01\ \mu\Omega^{-1}/\text{cm}^2$, $C_P = 30\ \mu F/\text{cm}^2$ and $G_P = 1000\ \mu\Omega^{-1}/\text{cm}^2$.

The values of $C_H$ and $G_H$ for the hydrocarbon region are completely * determined by the values of $C$ and $G$ for the whole film at very low frequencies (0.1—1.0 Hz). The possible range of values of $G_P$ and $C_P$ in fitting the experimental data to Eqns. 1 is very limited as has been shown previously (e.g. see Fig. 8, ref. 9 and Fig. 2, ref. 10). Usually it was possible to determine $C_P$ and $G_P$ for a given membrane, to an accuracy of ≈10%. The correlation ** between the experimental data and the theoretical values is most sensitive to the values of $C_H$ and $G_H$. Thus a 0.1% change in $C_H$ and a 1% change in $G_H$ produced a decrease of 0.1 in the correlation coefficient.

In the results shown in Fig. 1 note particularly the dramatic effect of indoleacetic acid on the dispersion in capacitance at higher frequencies (>10 Hz). This is due to a decrease in the surface layer conductance $G_\ell$ when indoleacetic acid is present. The dependence of the high frequency dispersion characteristics on the magnitude of $G_\ell$ is illustrated in Fig. 2.

*(b) Effect of KCl concentration on the penetration of indoleacetic acid*

It was found in earlier studies on unmodified bimolecular lipid membranes

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* For concentrations of KCl > 100 mM it was found that the conductance of the hydrocarbon region becomes large enough for the polar region to feature in the total impedance, even at very low frequencies.

** The correlation coefficient, $r$, is given by $r = (1 - \Sigma\, S_{th}^2/S_{ex}^2)^{1/2}$ where $S_{th}$ is the difference between the theoretical and mean experimental values at a given frequency. $S_{ex}$ is the standard deviation of the experimental points.

[9] that $C_H$ was largely independent of the concentration of KCl in the external solution. The conductance of the hydrocarbon region, however, as might be expected, did depend on the concentration of ions in the external solution. The conductance of the polar-head region was found to be weakly dependent on the KCl concentration. The polar-head region capacitance was not systematically dependent on the concentration of KCl in unmodified membranes. Similar results were obtained in the present study.

An example of the effect of indoleacetic acid on the dispersion characteristics in 100 mM KCl is shown in Fig. 3. Note the large effect of $10^{-6}$ M indoleacetic

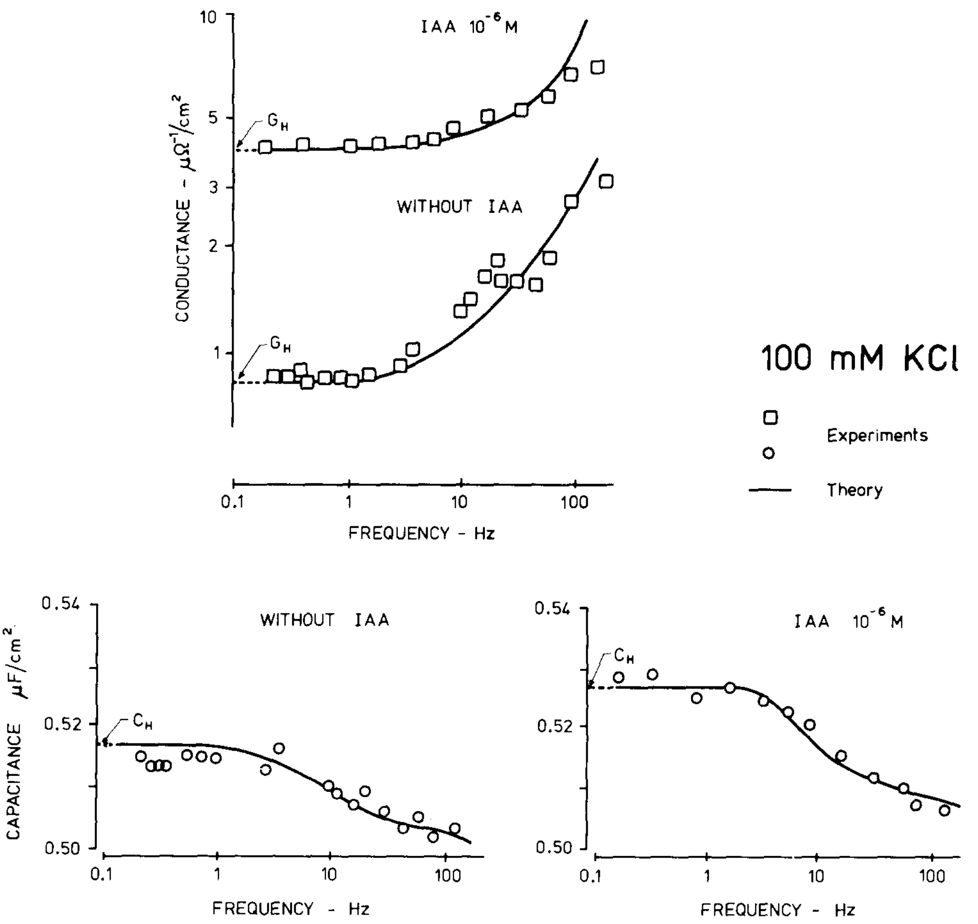


Fig. 3. Capacitance and conductance as a function of frequency for a bimolecular phosphatidylcholine membrane made in 100 mM KCl without indoleacetic acid and with $10^{-6}$ M indoleacetic acid. For clarity the results with and without indoleacetic acid for the capacitance are shown separately as they correspond very closely. The full curves are plots of Eqns. 1 with the values of $C_H$ and $G_H$ indicated on the diagrams. The other parameters used in the plots were: without indoleacetic acid, $C_P$ = 36 $\mu F/cm^2$, $G_P$ = 3000 $\mu\Omega^{-1}/cm^2$, $G_Q$ = 25 000 $\mu\Omega^{-1}/cm^2$; with $10^{-6}$ M indoleacetic acid, $C_P$ = 20 $\mu F/cm^2$, $G_P$ = 1200 $\mu\Omega^{-1}/cm^2$, $G_Q$ = 8000 $\mu\Omega^{-1}/cm^2$. Note that the conductance, $G_H$, of the hydrocarbon region in the presence of indoleacetic acid is much greater than for a membrane in the absence of indoleacetic acid. The polar-head region capacitance and conductance were substantially reduced in the presence of indoleacetic acid. IAA, indoleacetic acid.

acid on the conductance, $G_H$, of the hydrocarbon region. This was not observed in 1 mM KCl (see Fig. 1).

Thus when indoleacetic acid was added to the external solution the conductance $G_H$ of the hydrocarbon region increased and the conductance $G_\ell$ of the surface layer decreased. For a given indoleacetic acid concentration the magnitude of this effect moreover was strongly dependent on the concentration of KCl in the external solution. This is shown in Figs. 4 and 5 where $G_H$ and $G_\ell$ in the presence of $10^{-6}$ M indoleacetic acid are plotted as a function of KCl concentration. For comparison plots are also given for $G_H$ and $G_\ell$ in the absence of indoleacetic acid. It is evident that at low KCl concentrations indoleacetic acid had little effect on the conductance of the hydrocarbon region. The conductance of the surface layer, however, was reduced at low concentrations of KCl; in 1 mM KCl from a value of about 3000 $\mu\Omega^{-1}/cm^2$ when no indoleacetic acid was present to about 1100 $\mu\Omega^{-1}/cm^2$ in the presence of $10^{-6}$ M indoleacetic acid. As the KCl concentration was increased the effect of indoleacetic acid on the conductance of the hydrocarbon region increased while its effect on the surface layer conductance decreased. At 1000 mM the surface layer conductance was almost the same as for a bimolecular lipid membrane in the absence of indoleacetic acid while $G_H$ was increased about 13-fold. Concomitant with the increase in $G_H$ and decrease of $G_\ell$, when indoleacetic acid was present in the external solution, there was also a small but variable decrease in $G_P$, by a factor of $\approx 2$ (which was not dependent on the concentration of KCl). The average value of $C_P$ was reduced from $\approx 30$ $\mu F/cm^2$ to 18 $\mu F/cm^2$. The capacitance $C_H$ of the hydrocarbon region was relatively insensitive to the presence of indoleactic acid.

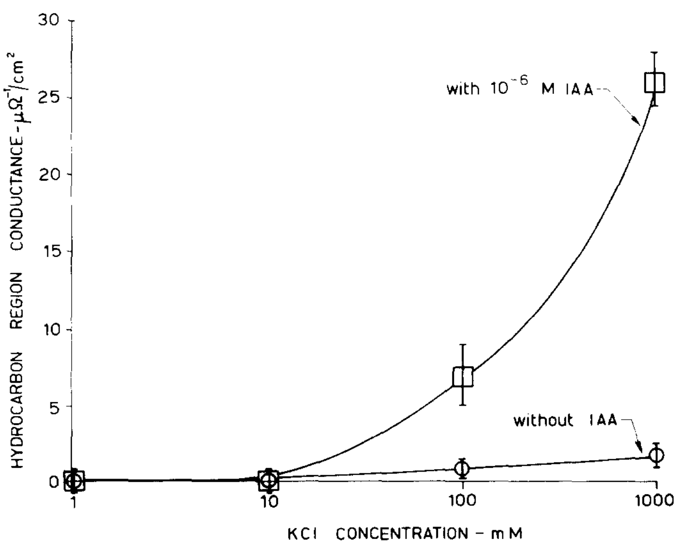


Fig. 4. The variation of the conductance $G_H$ of the hydrocarbon region of a phosphatidylcholine bimolecular lipid membrane as a function of the concentration of KCl, without indoleacetic acid (lower curve) and with $10^{-6}$ M indoleacetic acid (upper curve). The vertical bars indicate the standard errors (average of 15 measurements per point). IAA, indoleacetic acid.

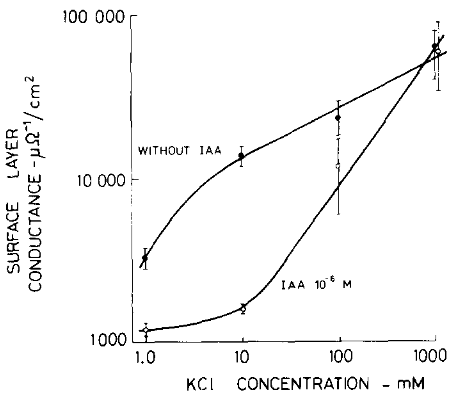


Fig. 5. The effect of concentration of KCl in the external solution on the conductance, $G_ℓ$, of the surface layer element in the unstirred solution layer without indoleacetic acid (upper curve) and with $10^{-6}$ M indoleacetic acid (lower curve). At low concentrations of KCl indoleacetic acid markedly reduced the conductance of the surface layer, presumably due to adsorption of indoleacetic acid onto the surface of the membrane. At high concentrations of KCl (>100 mM) $G_ℓ$ was so large that it was difficult to accurately determine its value from the dispersion characteristics obtained up to ≈100 Hz. IAA, indoleacetic acid.

## Discussion

At high concentrations of KCl, indoleacetic acid had a dramatic effect on the conductance of the hydrocarbon region of the bimolecular lipid membranes. Thus at 1000 mM KCl $G_H$ was increased in the presence of $10^{-6}$ M indoleacetic acid from a value of 2 $\mu\Omega^{-1}/cm^2$ to 26 $\mu\Omega^{-1}/cm^2$. Such an increase in the conductance of this phase could, in principle, result either from an increase in the ion mobilities (by a factor of ≈13) or from an increase in the ion concentrations in this region (that is a change in the ion partitioning into this phase from the external solution). An increase in the mobility of this order of magnitude could come about from changes in the packing of the hydrocarbon tails of the phosphatidylcholine molecules and/or fluidity of this region. Very small changes in the dielectric constant of this phase (insufficient to produce detectable changes in $C_H$), could also, through their effect on ion partitioning and hence concentration, account for the enhanced $G_H$.

The decrease in the polar-head capacitance when indoleacetic acid was present in the external solution could result from a decrease in the dielectric constant or an increase in the thickness of this region. The indoleacetic acid molecule has dimensions ≈0.4 nm × 0.9 nm × 0.9 nm (estimated from a molecular model). This is not greatly different from the polar-head portion of the phosphatidylcholine molecule. Absorption of indoleacetic acid into the polar-head region is feasible and could lower its dielectric constant (normally estimated to be ≈20, see ref. 9) by a displacement of water molecules. This would be consistent with the drop in the conductance $G_P$ which occurred when indoleacetic acid was present. Alternatively the absorption of indoleacetic acid may lead to an unfolding of the otherwise partially folded phosphate trimethylammonium dipole.

It appears, however, that the indoleacetic acid does not merely penetrate into the polar-head regions but also adsorbs onto the surfaces of the membrane. This is suggested by the decrease in the conductance of the surface layer.

As the KCl concentration increases, the effect of indoleacetic acid on $G_\ell$ decreases. In the light of this we would suggest that the adsorption of indoleacetic acid molecules onto the surface may be reduced. If hydrogen bonding

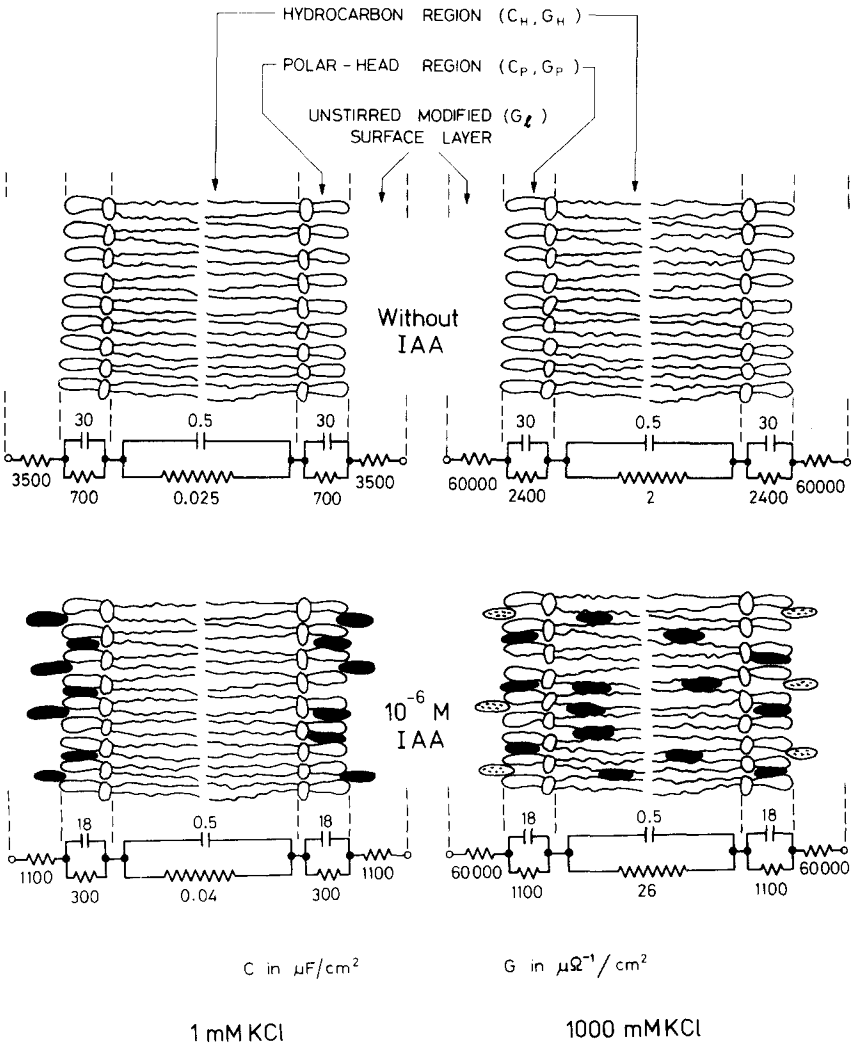


Fig. 6. A schematic diagram of the bimolecular lipid membrane without indoleacetic acid (upper figures) and with $10^{-6}$ M indoleacetic acid (lower figures) for external solutions containing 1 mM KCl and 1000 mM KCl. Typical values of the electrical parameters $C_H$, $G_H$, $C_P$, $G_P$ and $G_\ell$ from which the situations in each case were deduced are indicated on the equivalent circuit accompanying each scheme. The indoleacetic acid molecules (shown black) when the membrane was made in 1 mM KCl were located only in the polar-head regions and adsorbed onto the surface; $C_H$ and $G_H$ were largely unaffected by the presence of indoleacetic acid whilst $C_P$, $G_P$ and $G_\ell$ were very substantially reduced. At 1000 mM KCl concentration the indoleacetic acid molecules absorbed into the hydrocarbon region; $G_H$ then increased 13-fold, $C_P$ and $G_P$ remained markedly reduced and the surface layer conductance was almost unaltered by the indoleacetic acid. It is then not certain to what extent indoleacetic acid molecules are adsorbed on the surface (indicated in the diagram by dotting). IAA, indoleacetic acid.

features strongly in this adsorption a reduction in the H-bonding might then also be expected with increasing KCl concentration. The reduced surface absorption, however, is associated with an increase in the conductance of the hydrocarbon region (see Fig. 4). Thus we are led to conclude that as the KCl concentration increases the indoleacetic acid penetrates further into the membrane; into the hydrocarbon region leaving the surface largely in its unmodified state *. The scheme with the situation at 1 mM and 1000 mM KCl, together with summaries of the electrical parameters from which these deductions are made, are shown in Fig. 6.

On the basis of our findings, the effect of indoleacetic acid on the ion transport and permeability of cell membranes, might be expected to be dependent on the KCl concentration. Indeed such a dependence has been reported [13].

Ion concentrations in the unstirred layers near membranes may be significantly perturbed due to diffusion effects related to either unequal transport numbers (i.e. concentration polarisation [14] and/or the activity of ion pumps). When indoleacetic acid is present our results would suggest that such changes in ion concentration would then be reflected in the penetration of indoleacetic acid. This would be quite apart from the direct effect of ion concentration in the bulk of the external (and internal) medium.

In general, concentration polarisation effects in unstirred layers near cell membranes have only been considered in relation to their direct influence on the conjugate fluxes. The results reported here, however, suggest that concentration polarisation in the unstirred layers may also affect directly the penetration or location within the membrane of some hormones or inhibitors. The latter can influence other ion transport systems and hence a variety of such transport mechanisms may become interdependent. In regard to this it is interesting to note that the effects we report for bimolecular lipid membranes do occur at concentrations of indoleacetic acid which also affected the electrical properties and the influx and efflux of $K^+$ in *Valonia utricularis* [7,8]. In these experiments net fluxes of $K^+$ occur which could well perturb the ionic concentrations at the membrane surface thus influencing the action of indoleacetic acid.

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* When $G_Q$ is very large, as it was in membranes made in 1000 mM KCl, it is difficult from measurements <100 Hz, to determine $G_Q$ with sufficient accuracy from the dispersion curves to allow us to determine whether the presence of indoleacetic acid then substantially modifies this surface layer, see also Fig. 2.

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