The Effect of Drugs on Cell Membranes with Special Reference to Local Anaesthetics*

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Increasing knowledge about the cell and its functions has made it clear that the cell membrane and the membranes inside the cell, in the mitochondria, the microsomes, the cell nucleus and in the cytoplasm are not only diffusion barriers which separate water phases but that a large number of very important processes takes place at these membranes. This knowledge has raised a number of questions.

The Structure of the Cell Membrane

First of all, what is the structure of the membranes?

The exact structure is unknown but it seems that lipids are an essential part of the membranes and that these lipids are arranged in a bimolecular layer where the lipophilic groups face each other while the hydrophilic groups turn to the water phases on the two sides of the membrane. On the lipids on both sides of the membrane there seems to be adsorbed a monolayer of partly unfolded proteins (Danielli, 1958) (Fig. 1).

The Relation between Structure and Function

Secondly, what is the significance of the physical structure of the membranes in relation to the processes which take place in them?

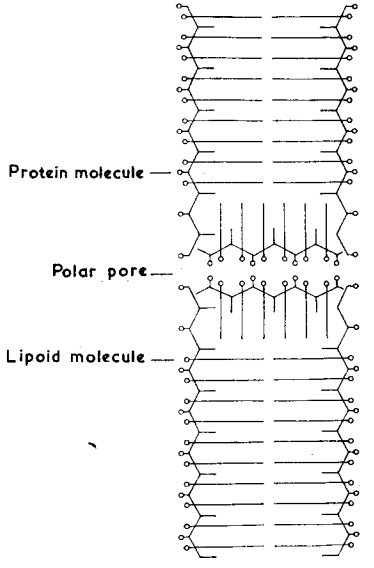

Fig. 1. Model of a cell membrane showing a polar pore (modified from Danielli, 1958).

* Based on a lecture at the joint meeting of the British Pharmacological Society and the Scandinavian Pharmacological Society, Copenhagen, 1960.

Are the membranes only the building stones on which the active elements are placed, or are the membrane elements part of the system? What is the difference between an enzymatic process which takes place at an interface and one which takes place in a solution? Are there other ways

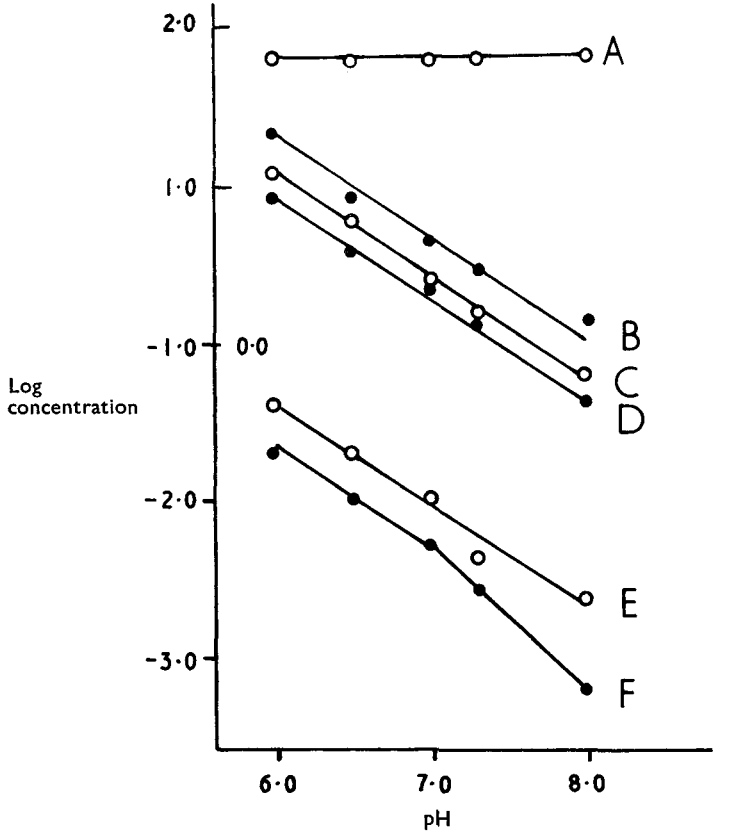

FIG. 2. The dependence of the minimum blocking concentrations on the pH (Skou, 1954a). Ordinate: the logarithms of the minimum blocking concentrations in mM. The lower scale applies to amethocaine (E) and cinchocaine (F), the upper scale to butanol (A), procaine (B), cocaine (C), tropacocaine (D).

at the interface by which the process can be influenced or controlled which differ from those in a solution? And if so, what are they?

Function and Drug Action

Thirdly, how must we interpret the effects that drugs have on the reactions taking place at an interface? Is the effect of a drug at an interface different from that found in a solution? Will a drug by altering the structure of a membrane be able to influence indirectly the chemical processes therein?

It is not possible at the present moment to give an answer to these questions, but in the following pages will be described some experiments

with local anaesthetics which show one of the ways by which it may be possible to get information about these problems.

The Effect of Local Anaesthetics

Most of the local anaesthetics in use are tertiary amines and their pK value is of such an order that the drugs at a physiological pH will be in the solution partly as the undissociated base and partly as the cation. The physicochemical properties of these two components are different. The cation is much more soluble in water than in lipids, while the undissociated base is much more lipid than water soluble. It is therefore reasonable to assume that the two components of the molecule have different pharmacological effects.

The Undissociated Base

For local anaesthetics which are tertiary amines the minimum concentration which can block nerve conduction decreases with increasing pH

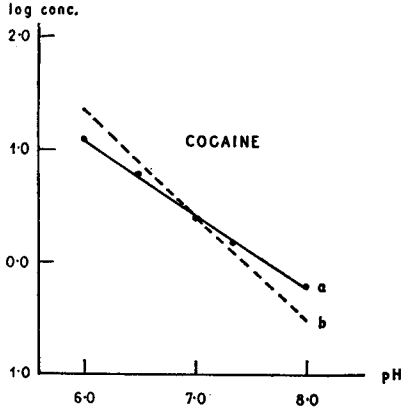

FIG. 3. Cocaine, the dependence of the minimum blocking concentration on pH. Ordinate: Curve 'a', logarithms of the minimum blocking concentrations in mM. Curve 'b' indicates the concentrations of cocaine which at the different values of pH give the same concentration of undissociated base in the solution as the minimum blocking concentration of cocaine at pH 7·0 (Skou, 1954b).

(Fig. 2). For butanol which also can block nerve conduction the minimum blocking concentration is independent of pH. Since butanol is undissociated this indicates that a change in the hydrogen ion concentration does not in itself influence the excitability of the nerve. The change in minimum blocking concentration of the tertiary amines must therefore be due to the change in the ratio between cation and undissociated base.

With an increase in pH the amount of undissociated base increases while the amount of cation decreases. The increase in blocking potency with increasing pH therefore makes it reasonable to assume that it is the undissociated base which is the blocking agent. If this is correct the minimum blocking concentration calculated as the concentration of undissociated base must be the same at different values of pH. But as seen from Fig. 3 this does not seem to be the case. The line 'b' indicates

the concentrations of cocaine which in solution give the same concentration of undissociated base at the different values of pH, while the line 'a' is the estimated minimum blocking concentration of cocaine at different values of pH (*cf.* Fig. 2). The slope of the two lines is similar but not identical, which means that the minimum blocking concentration of undissociated base varies with pH; it increases with increasing pH. If, however, as pointed out by Shanes (1958), an activity correction is applied to the estimated concentrations of the undissociated base, the minimum blocking concentrations at different pH correspond to the same activity of base in solution. That means that the slope of the corrected

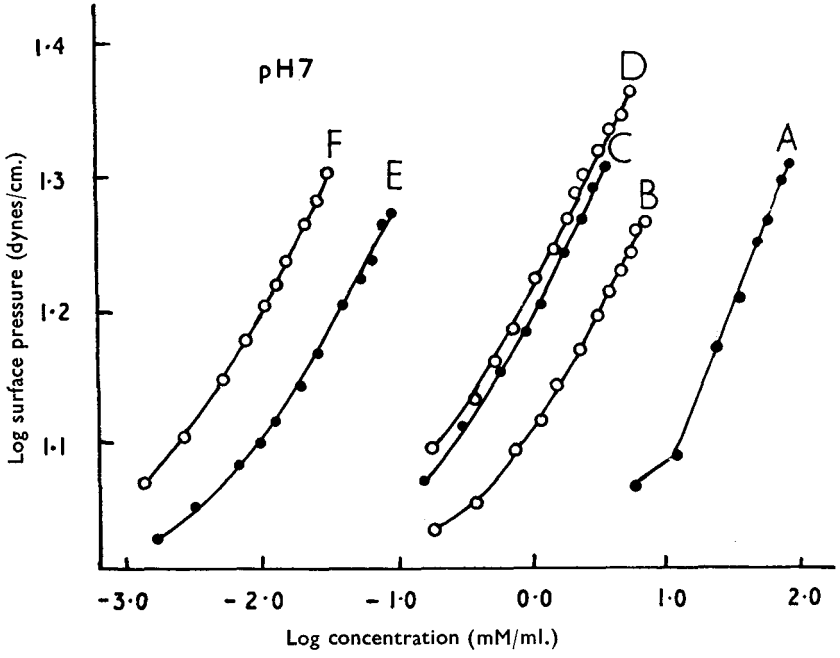

FIG. 4. Curves showing the relation of pressure to concentration for: butanol (A), procaine (B), cocaine (C), tropacocaine (D), amethocaine (E), cinchocaine (F). Monolayer of nerve-tissue lipids in a Ringer phosphate buffer pH 7·0 (Skou, 1954c).

curve for the estimated minimum blocking concentrations will be identical with the slope of 'b' and that it is the undissociated base which is the blocking agent.

The undissociated base of a local anaesthetic is more lipid than water soluble. It is therefore reasonable to assume that in the cell, it will be found in a higher concentration in the lipid phase in the cell membrane than in the water phase.

When an extract of lipids from peripheral nerves is spread on a water surface where the area of the surface is large enough compared to the amount of lipids, the lipid molecules will form a monomolecular layer. In this monolayer the molecules will be oriented in relation to the water

surface in the same way as it is assumed that they are in the cell membrane (*cf.* Fig. 1). Such a monolayer thus forms a model of the interface between water and lipids in the membrane and it may therefore be possible by the help of this model to gather information about the effect of the local anaesthetics on the cell membrane.

The area of the monolayer can be varied by a barrier which is placed across the trough in which the water surface is at the same level as the side of the trough. When the area is decreased the surface pressure is increased. The surface pressure is identical with the decrease of the surface tension of the water surface due to the monolayer.

In the investigations on the effect of local anaesthetics on this model of a cell, lipids extracted from peripheral nerves were spread on the surface

TABLE I

THE RATIOS BETWEEN THE MINIMUM BLOCKING CONCENTRATIONS AND BETWEEN THE INCREASES IN PRESSURE IN THE MONOLAYER, PH 7·0. IN COLUMNS 3 AND 5, PROCAINE HAS BEEN TAKEN AS 1 (SKOU, 1954 C).

	Minimum blocking concentration	Relative blocking potency	Increase in pressure in monolayer	Relative increase in pressure
	mM/l.		dynes/cm.	
Procaine	4·6	1	7·0	1
Cocaine	2·6	1·8	8·9	1·3
Tropacocaine	2·2	2·1	9·4	1·3
Amethocaine	0·01	460	2·5	0·4
Cinchocaine	0·005	920	3·8	0·5
Butanol	68	0·07	9·8	1·4

of a Ringer's solution, pH 7·0. The area of the monolayer was adjusted so the pressure in the monolayer was 10 dynes/cm. This value was arbitrarily chosen. The local anaesthetics were injected in the water-phase beneath the monolayer and after a thorough mixing the change in surface pressure due to the penetration of the local anaesthetic was measured at a constant area of the monolayer.

The results of these experiments are shown in Fig. 4, and in Table I is shown the pressure increases in the monolayer produced by a concentration in the water phase beneath the monolayer equal to the minimum blocking concentration of the local anaesthetic.

It is seen from Fig. 4 and Table I, Column 3, that the order in which the local anaesthetics increase the pressure in the monolayer is the same as the order of their blocking potency. As shown in Table I the minimum blocking concentrations of the local anaesthetics vary from 4·6 to 0·005 mM/l., that is, by a factor 1:920; if butanol is included the factor is 1:13,500. Considering this, the pressure increase produced by the minimum blocking concentrations, even if it varies with a factor of 1:3, seems to be of the same size for all the agents.

In Fig. 5 curve 'm' shows the concentrations of cocaine that at pH 6·0 and 7·5 produce the same increase in pressure in the lipid monolayer, 8·9 dynes/cm., as the minimum blocking concentration 2·6 mM/l. at pH 7·0. The curve 'a' indicates the slope of the curve for the dependence of the minimum blocking concentrations on the pH (*cf.* Fig. 2). The

similarity between the slope of these two lines indicates that the ability of the local anaesthetic to increase the pressure in the monolayer depends on pH in a manner which is similar to its ability to block nerve conduction.

In Table II is shown the results of the investigations of a number of drugs, which like the tertiary amines, are able to block the impulse conduction in peripheral nerves. They are all undissociated in aqueous solution. Also it was found that when these drugs were injected in the water phase below a monolayer of nerve lipids, they gave an increase in pressure in the monolayer. And as it is seen from the Table the minimum blocking concentrations of these drugs gave pressure increases in the monolayer which were of the same size.

The experiments on the monolayer thus show (1) that the order of the ability of the drugs to increase the pressure in a monolayer of nerve tissue

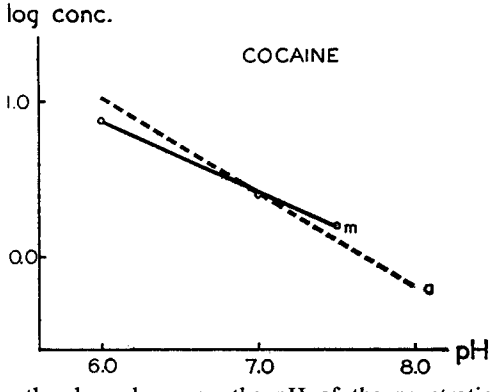

FIG. 5. Cocaine, the dependence on the pH of the penetration (Skou, 1954c). Curve 'm', logarithms of the concentrations which produce an increase in pressure of 8·9 dynes/cm. Curve 'a' indicates the slope of the curve for the dependence on the pH of the minimum blocking concentrations.

lipids follows that of their ability to block nerve conduction, (2) that the minimum blocking concentrations of the drugs give increases in the pressure in the monolayer which are of the same size, and (3), that the ability of the tertiary amines to increase the pressure in the monolayer depends on pH in a manner similar to their ability to block nerve conduction.

It seems therefore reasonable to conclude that there is a correlation between the ability of the drugs investigated to block nerve conduction and their ability to increase the pressure in a monolayer of nerve tissue lipids.

This indicates (1) that the site of the blocking action is a lipid containing membrane and (2) that the effect is due to the physical changes which are produced by the penetration of the drugs into the membrane.

This view has found strong support in the results of experiments by Shanes and Gershfeld (1960) on stearic acid films. It was found that labilisers such as some veratrum alkaloids and stabilisers such as procaine, which is known to have an antagonistic effect on the nerve membrane, also have antagonistic effects on the monolayer. Labilisers decreased the

spreading force while stabilisers, in accordance with the results from the experiments on the monolayer of lipids from nerve tissue, increased the spreading force.

The nerve impulse is caused by a transient increase in the permeability of the axon membrane to Na^+ (Hodgkin, 1951). It is therefore reasonable to assume that the membrane at which the local anaesthetics exert their blocking action is the axon membrane, and that it is the physical changes in the lipid part of the membrane due to the penetration of the drugs that prevents the increase in permeability to Na^+ and thereby block the impulse.

According to Danielli (1958), the ions move through pores in the membrane and he has suggested that the pore is the space between two protein lamellae which are placed with the polar groups facing each other

TABLE II*

THE PRESSURE INCREASE PRODUCED BY THE MINIMUM BLOCKING CONCENTRATIONS OF DRUGS IN A MONOLAYER OF NERVE TISSUE LIPIDS AT PH7

1	2 Minimum blocking concentrations mM/l.	3 Corresponding pressure increases in monolayer dynes/cm.	4 Concentrations giving a pressure increase of 9·3 dynes/cm. mM/l.	5 Relative blocking potencies	6 Relative penetrating potencies
Propanol	218	8·5	240	1·0	1·0
Isopropanol ..	351	8·0	438	0·6	0·6
Butanol	68	8·2†	80	3·2	3·0
Pentanol	21	10·0	19	10·4	12·6
t-Pentanol ..	81	8·9	85	2·7	2·8
Menthol	0·58	11·8	0·37	376	649
Thymol	0·22	9·8	0·20	991	1200
β-Naphthol ..	0·30	9·5	0·29	727	828
Average		9·3			

* Skou, 1958.

† In previous studies, see Table I, the corresponding figure was found to be equal to 9·7 dynes/cm. The explanation presumably is that the vapour pressure of butanol above the monolayer was too high in the earlier experiments, the atmosphere being saturated with butanol.

while the non-polar groups face the lipid part of the membrane (*cf.* Fig. 1).

What makes the pores in the membrane discriminate between Na^+ and K^+ is unknown as is also the mechanism that underlies the change in permeability of Na^+ during the impulse. But whatever the mechanisms it is reasonable to assume, as suggested by Danielli, that proteins play an important role.

How then can a drug which penetrates into the lipid part of the membrane influence this process, or, to put it in another way, how can a drug which penetrates into the lipid part of the membrane influence the proteins at the interface in a pore in the membrane?

At an interface between water and lipids a protein will be more or less unfolded and the degree of unfolding will depend on the interfacial tension. Furthermore, the side chains of the protein will be oriented relative to the interface, and the orientation will depend among other factors such as pH, temperature and ions in the water phase, on the

lipids; the orientation of the lipophilic side chains is influenced by their affinity for the lipophilic parts of the lipids and the orientation of the hydrophilic side chains is effected by their interaction with the hydrophilic groups of the lipids. A change in the structure of the lipids may therefore lead (1) to a change in the degree of unfolding of the protein and (2) to a change in the orientation of the side chains.

In order to see whether a change in one of these factors might influence the activity of a protein, a technique was elaborated (Skou, 1959) which made it possible to investigate how the activity of an enzyme at an interface depends on the degree of unfolding and on the orientation of the side chains of the protein.

When a protein is spread on aqueous surface, an unfolding of the molecule takes place (Bull, 1947). The rate of unfolding depends on a

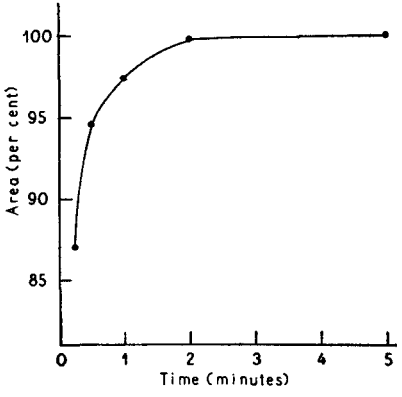

FIG. 6. Area of the surface-spread protein after the protein has been allowed to spread against zero film pressure for varying periods after the end of application. The area is expressed as a percentage of the area at complete unfolding (Skou, 1959).

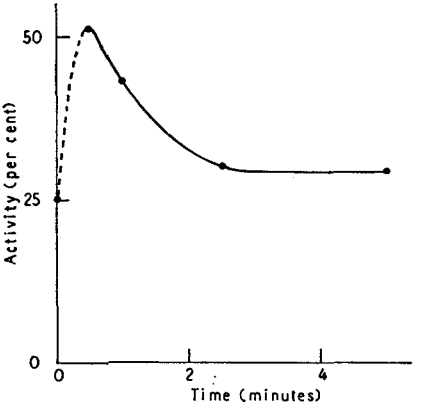

FIG. 7. Acetylcholinesterase activity of the surface-spread protein after it has been allowed to spread against zero film pressure for varying periods after the end of the application. The activity was determined at a surface pressure of 10 dynes/cm. and is expressed as a percentage of the activity of the same amount of acetylcholinesterase in solution (Skou, 1959).

number of factors such as the temperature, pH, and ionic composition of the fluid. In addition, it depends on the surface pressure; the higher the pressure the more difficult is the unfolding and at a sufficiently high surface pressure unfolding is prevented.

Complete unfolding takes a certain time; for the protein investigated, acetylcholinesterase, it took 2 min. (Fig. 6). At any degree of unfolding the unfolding could be stopped by applying a surface pressure of 7 dynes/cm. or greater. It was therefore possible to obtain the protein at any degree of unfolding by applying a surface pressure of 7 dynes/cm. at different time intervals after the application of the protein to the surface.

The orientation of the side chains of a protein on a water surface in relation to the surface varies with the surface pressure (Bull, 1947; Davies,

1953; Ellis and Pankhurst, 1954; Hughes and Rideal, 1932). At zero film pressure the side chains will lie flat in the surface. If the pressure is increased, the polar groups are orientated towards the water phase, while the non-polar groups will be raised in the air. It is therefore possible to vary the orientation of the side chains by applying surface pressures of varying magnitude.

The enzyme investigated was acetylcholinesterase purified from tissue from *Electrophorus electricus* (Rothenberg and Nachmansohn, 1947). The activity of this enzyme spread on the surface could be measured by measuring the breakdown of acetylcholine in the water phase beneath the

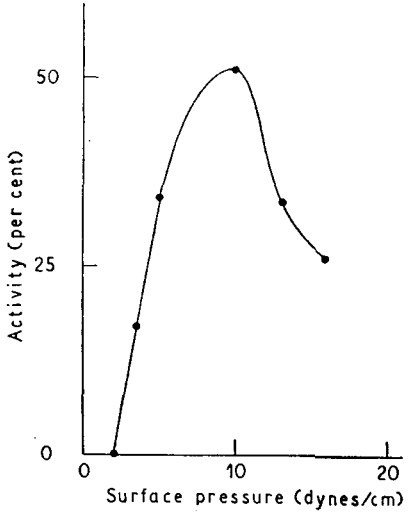

FIG. 8. Acetylcholinesterase activity of the surface-spread protein at varying surface pressures. In all experiments the protein was allowed to spread against zero film pressure for 30 sec. The activity is expressed as a percentage of the activity of the same amount of acetylcholinesterase in solution (Skou, 1959).

surface. The activity of the enzyme at the surface is expressed as the percentage of the activity of the same amount of acetylcholinesterase in solution.

As is seen from Fig. 7, the activity of the enzyme varies with the time the protein has been allowed to unfold at the surface, that is, with the degree of unfolding (*cf.* Fig. 6). At complete unfolding and at a surface pressure of 10 dynes/cm. the enzyme still has about 29 per cent of the activity of the enzyme in solution.

In Fig. 8 is shown how the activity of the enzyme at the surface varies with the surface pressure at a given degree of unfolding. In all these experiments the protein was allowed to spread for 30 sec. against zero film pressure. It appears that maximum activity, 51 per cent, is attained at a pressure of 10 dynes/cm. and that an increase of the pressure from 10 to 16 dynes/cm. gives a decrease in activity from 50 per cent to 26 per cent while a decrease in pressure to 2 dynes/cm. results in complete abolition of the activity. It should, however, be noted that unfolding at

pressures ranging from 2 to 5 dynes/cm. is somewhat greater than at higher pressure, since, as previously mentioned, some further unfolding will take place after adjustment at these pressures. The values found are accordingly a little too low.

Experiments similar to those shown in Fig. 8 were also performed with a spreading time of 5 min., that is, at complete unfolding at the surface (*cf.* Fig. 6). Under these conditions, maximum enzyme activity, 29 per cent, was also found at a pressure of 10 dynes/cm.; at 2 dynes/cm. the activity was zero.

It is seen from these experiments that both a change in the degree of unfolding and in the orientation of the side chains will give a change in activity, and of these two factors the variation in the orientation of the side chains give the greatest change in activity.

As mentioned above, the orientation of the side chains of a protein at an interface between water and lipid among other factors depends on the interactions between the side chains and the lipophilic and hydrophilic part of the lipids. It is therefore reasonable to assume that a drug which penetrates into the lipid part of a membrane may indirectly, due to its effect on the lipids, influence the orientation of the side chains of proteins at the interface. This may, according to the results of the experiments presented above, lead to a change in the activity of processes in which the proteins at the interface participate.

Eisenman Rudin and Casby (1957a) have been able to make glass electrodes which have different sensitivities for Na^+ and K^+ and they (1957b) (see also Rudin and Eisenman, 1959; Isard, 1959) have put forward a theory according to which the different sensitivities of a system, living or non-living, to alkali metal ions are due to differences in electrostatic field strengths of the negatively charged groups in the system. At low negative electrostatic field strength the sensitivity for K^+ is higher than for Na^+, at high negative electrostatic field strength the sensitivity for Na^+ is higher than for K^+.

In a membrane the electrostatic field strength of the fixed negatively charged groups at the interface is determined by the overlapping of the electric fields of the positive and negative charged groups on the lipids as well as on the side chains of the proteins. The negative electrostatic field strength of the charged groups on the proteins is furthermore determined by the distribution of the electrons along the protein chain.

In a pore in a membrane both the overlapping of the electric fields of the charged groups and the distribution of electrons along the protein chain may be influenced by the potential across the membrane. At the resting potential across the nerve membrane the overlapping of the charged groups and the distribution of the electrons may be such as to give a low negative field strength of the fixed anions and that means, according to the theory of Eisenman and others (1957b) a high permeability of the pore to K^+ and a low permeability to Na^+. At a lowering of the potential the overlapping of the charged groups and the distribution of electrons may be shifted so as to give an increase in the negative electrostatic field strengths, that is an increase in permeability to Na^+.

Local anaesthetics, due to their effect on the lipids, may indirectly influence the orientation of the side chains of the proteins in the interface. This may change the overlapping of the electric fields of the charged groups at the interface in such a way that the depolarisation of the membrane, even if it gives a change in the distribution of the electrons along the protein chain, cannot lead to that increase in negative electrostatic field strengths which is necessary to increase the permeability of the pore to Na^+; that is the nerve impulse is blocked.

The Cation

The cationic part of the molecule of a local anaesthetic also has an effect on a cell membrane and as will be seen from the following this effect seems to be to open a membrane to Na^+ (Skou and Zerahn, 1959).

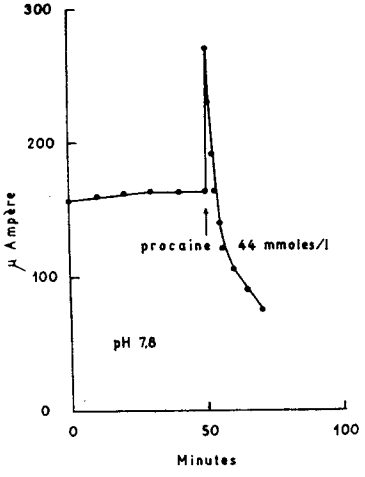

FIG. 9. The short circuit current for an isolated frog skin before and after the addition of procaine to the Ringer solution bathing the outside of the skin. Procaine concentration 44 mM, pH 7·8 (Skou and Zerahn, 1959).

The frog skin is able to transport Na^+ from the outside of the skin to the inside. This transport can be measured by the current which is necessary to short-circuit the potential across the skin. (Koefoed-Johnsen and Ussing, 1958; Ussing and Zerahn, 1951).

When a local anaesthetic, for example, procaine, is added to the solution bathing the outside of the frog skin at pH 7·8 the short circuit current, that is the transport of Na^+, is first increased but shortly after again decreases to a value which is lower than before the addition (Fig. 9).

If the experiment is repeated at pH 6·0 where practically all the procaine is in the form of the cation it can be seen that the increase in current is not followed by a decrease (Fig. 10).

At pH 10·0, where nearly all the procaine is as the undissociated base addition of procaine to the outside gives no increase in current, only a decrease (Fig. 11).

If the tertiary procaine is converted to a quaternary drug by adding an extra CH_3 group on the amine, that is to change it to a drug which at

any pH is in the cationic form, this drug added to the outside of the skin always gives an increase in the short circuit current independent of the pH in the solution. In Fig. 12 is shown the effect at pH 7·8 (*cf.* Fig. 9).

Besides procaine, a number of other local anaesthetics have been tested. all were tertiary amines and for all of them it was found that in the cationic form they increased the active transport of Na^+ across the frog skin when

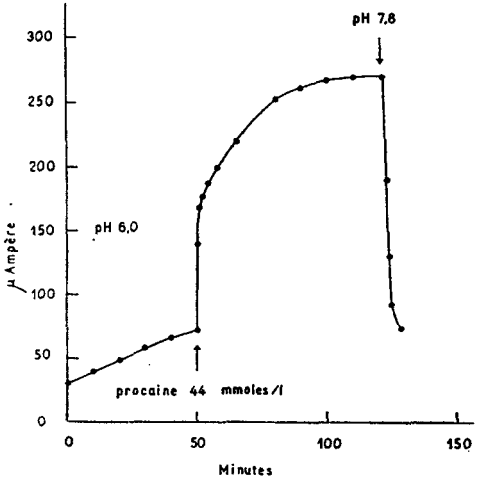

FIG. 10. The short-circuit current for an isolated frog skin before and after the addition of procaine to the Ringer solution bathing the outside of the skin. Procaine concentration 44 mM, pH 6·0 (Skou and Zerahn, 1959).

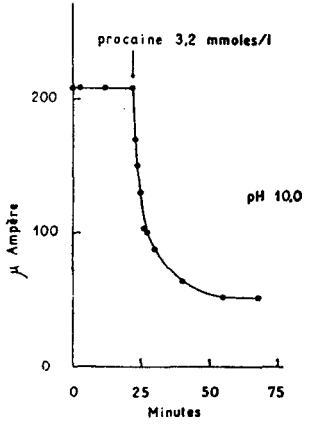

FIG. 11. The short-circuit current for an isolated frog skin before and after the addition of procaine to the Ringer solution bathing the outside of the skin. Procaine concentration 3·2 mM, pH 10·0 (Skou and Zerahn, 1959).

they were added to the outside solution. As undissociated bases they inhibited the active transport.

Added to the solution bathing the inside of the skin, the cationic form of the drugs have no effect while the undissociated base inhibits the active transport of Na^+.

The stimulating effect is not only limited to local anaesthetics, but a number of widely different drugs have the same effect. Common for all these drugs are (1) that they are amines, (2) that they must be added to the solution bathing the outside of the skin and (3) that they must be present in ionised form in the solution to exert the effect.

The stimulating effect seems to be independent of whether the drugs are primary, secondary, tertiary or quaternary amines. The effects of tertiary procaine and of quaternary "procaine" were of the same order of magnitude, and so were the effects of the primary amine, amphetamine, and the secondary amine, methamphetamine.

On the other hand, the effect seems to depend on the molecule to which the amino group is linked; it was found that the effect of the quaternary amine, acetylcholine, was much less than that of the quaternary "procaine".

Koefoed-Johnson and Ussing (1958) showed that the potential across the frog skin varied with the concentrations of Na^+ and K^+ on the two sides of the skin in such a manner that it must be assumed that the frog skin contains two functional membranes; an outer membrane, which is specifically permeable to Na^+ but not to the other cations, and which probably corresponds to the surface of the epithelial cells, and an inner membrane, which is specifically permeable to K^+, and which is probably situated at the inward-facing membrane of the stratum germinativum. These authors assume that the active transport of Na^+ is localised to the

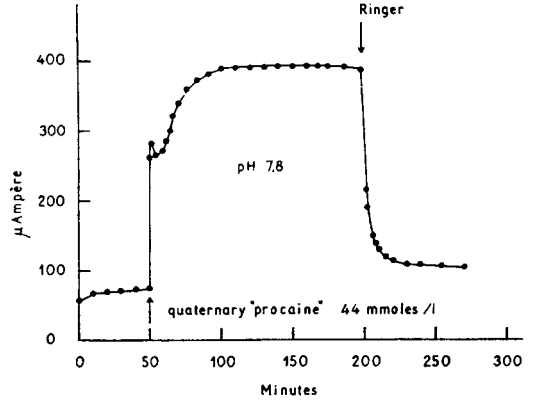

FIG. 12. The short-circuit current for an isolated frog skin before and after addition of quaternary "procaine" to the Ringer solution bathing the outside of the skin. Concentration of quaternary "procaine" 44 mM, pH 7·8 (Skou and Zerahn, 1959).

innermost of these two membranes, while no active transport occurs through the outer membrane. If transport through the skin is to take place, Na^+ must be transported passively through the outer membrane into the epithelial cells, from which active transport takes place through the inner membrane. This hypothesis has been supported by work by Engbæk and Hoshiko (1957) and by Schmidt (1960) who showed that the total potential across the epithelial layer is the sum of two potentials.

In experiments with giant axons, Hodgkin and Keynes (1956) showed that the active transport of Na^+ out of the nerve increased proportionally to the intra-axonal concentration of Na^+. If, similarly, the active transport of Na^+ from the epithelial cells of the frog skin to the fluid inside is proportional to the intracellular Na^+ concentration, an increase in the influx of Na^+ from the solution outside into the epithelial cells must result in an increase in active transport.

It is the ionized amines that are capable of increasing the active transport of Na^+, but only when they are added to the outside solution. Since the ionized amines do not pass through the skin, their point of attack is presumably the outermost of the two membranes in the frog skin, and their effect may consist in increasing the permeability of this membrane to Na^+. Since there is an electrochemical gradient for Na^+ from the solution outside to the epithelial cells when this solution is a Ringer

solution, an increase in the permeability of the membrane to Na^+ leads to an increase in the Na^+ influx and hence in the Na^+ concentration in the epithelial cells. As mentioned above this must be assumed to produce an increase in the active transport of Na^+ across the inner membrane.

This hypothesis about the effect of the ionized amines may be supported by the fact that quaternary amines are able to increase the permeability to Na^+ of the membranes of the muscle end-plate (*cf.* Riker, 1953) and of the electroplax from *Electrophorus electricus* (Altamirano, Coates, Grundfest and Nachmansohn, 1955; Schoffeniels and Nachmansohn, 1957).

We have made no experiments on the effect of the cationic form of the local anaesthetics on the nerve membrane.

The experiments with local anaesthetics, which are tertiary amines thus show that the effect of the undissociated base and of the cation are different but that both effects are due to an effect on a cell membrane.

The effect of the undissociated base is to block the increase in permeability of the nerve membrane to Na^+ and this effect seems to be due to a penetration of the molecule into the lipid part of the nerve membrane. The effect of the cation seems to be to increase the permeability of a membrane, the outer membrane of the frog skin, to Na^+.

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