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STIMULATION OF CHLORIDE TRANSPORT BY FATTY ACIDS IN CORNEAL EPITHELIUM AND RELATION TO CHANGES IN MEMBRANE FLUIDITY

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Summary

The effect of altering cell membrane lipids on ion transport across isolated corneas was studied. Corneas mounted in Ussing-type chambers showed a rapid increase in short-circuit current following treatment with a variety of unsaturated fatty acids of varying chain length and unsaturation. Measurements of membrane fluidity which utilize immunofluorescence labelling of membrane proteins showed corneal epithelial cell membranes to be significantly more fluid following linoleic acid tratment. Uptake studies indicate rapid incorporation of [^{14}C]linoleic acid into corneal cell membranes. Highly unsaturated fatty acids were found to have the greatest ability to stimulate chloride transport. Saturated fatty acids were tested and were found to have no effect on chloride transport at any concentration. It is proposed that unsaturated fatty acids activate chloride transport by increasing membrane lipid fluidity. The relationship of these parameters is discussed in terms of a mobile receptor model. We speculate that an increase in membrane lipid fluidity promotes lateral diffusion of membrane receptor proteins and enzymes, increasing protein-protein interactions within the membrane, ultimately resulting in the enhancement of cyclic AMP synthesis.

Introduction

Over the past few years a large number of observations have confirmed that the cell membrane is a fluid, dynamic structure. It has also become clear that membrane components such as receptors and membrane-bound enzymes have the ability to undergo topographic redistribution. These membrane proteins are thought to be normally free to diffuse or migrate laterally in the plane of the

membrane, the rate of diffusion being restricted by several factors, one of which is viscosity of the lipids composing the membrane [1]. Membrane fluidity may be considered to be the inverse of viscosity; therefore, a very fluid membrane should have a high rate of protein diffusion and low viscosity. It is believed that the lateral diffusion of proteins within the plane of the membrane may play a significant role in a variety of membrane-mediated biological and biochemical functions [2–4].

Certain membrane-bound enzymes have the specific requirement that membrane phospholipids be in a fluid state to permit full activity [5,6]. Increases in lipid fluidity have been shown to activate ATPase [7], and adenylate cyclase activity [8]. Changes in membrane lipid fluidity have also been shown to be associated with malignant disorders [9], as well as the transformation in culture of fibroblastic cell lines [10–12].

Protein diffusion within the plasma membrane normally may be regulated in part through the cell's ability to control membrane lipid viscosity, by adjusting the ratio of saturated to unsaturated fatty acids, a process termed homeoviscous adaptation [13,14]. It is possible, however, to insert specific fatty acids by substitution into cell membrane phospholipids, and to study the resulting changes in membrane properties [15,16]. For example, the addition of certain fatty acids has been shown to induce cell fusion in hen erythrocytes [17]. Also, studies utilizing phospholipid vesicles (liposomes) showed greater increases in cell fusion in mammalian cell lines with vesicles in the fluid state than when vesicles in the solid phase were used [18]. In another study, Schaeffer and Curtis [19], showed that unsaturated fatty acid supplements added to L929 fibroblast cultures caused fluidization of the cell membrane and simultaneously a decreased ability of these cells to adhere to the substratum. However saturated fatty acids had the opposite effect; that is, the increased membrane viscosity while increasing cell adhesiveness [19].

Chloride active transport is a membrane-mediated phenomenon occurring in a variety of cells and tissues. Previous research in the cornea of both frogs and rabbits, and more recently in the opercular epithelium of fish, clearly indicates that the chloride pump is responsible for the current carried across these epithelia and that its function is to produce osmotic gradients for dehydration of swollen corneas and osmoregulation in the fish [20–26]. Chloride transport is regulated by the catecholamines in the corneal epithelium as shown by Chalfie et al. [23], and by Klyce et al. [24]. The mechanism of action of the catecholamines is through activation of adenylate cyclase in the cell membranes of the epithelia, and consequent increase in cyclic AMP in the intracellular compartment. A variety of drugs acting through the cyclic AMP system have been shown to stimulate chloride transport in the cornea [27].

In this paper we present evidence that chloride transport across corneal epithelium can be modified by fatty acid-induced changes in membrane fluidity. The ability to manipulate membrane lipid composition represents a useful tool with potential for furthering the understanding of membrane function.

Materials and Methods

Chloride transport

Frogs (*Rana catesbeiana*) and albino rabbits were killed and corneas immedi-

ately dissected and mounted between the two chamber halves of Ussing-type chambers equipped with bubble lifts for fluid circulation and aeration, and polyethylene/agar/Ringer's bridges for the measurement of transcorneal potential difference and for passing the short-circuit current as described previously [20]. Frog or mammalian balanced Ringer's solution bathes both corneal surfaces. Water-jacketed rabbit corneal chambers were maintained at 37°C during the experiment, while frog chambers were at room temperature, thus approximating the normal physiologic temperature characteristic of each animal.

The short-circuit current, which is proportional to the net ion transport across the cornea, is obtained by use of automatic voltage clamp units, and voltage clamping to zero. The short-circuit current is continuously monitored during the experiment by means of a two-channel chart recorder. Transcorneal potential difference is intermittently monitored during the course of the experiment.

Unidirectional active $^{36}Cl^-$ flux determinations on paired corneas were done in the chambers described above. In summary, $^{36}Cl^-$ is added to the endothelial side of the chamber, the corneas are short-circuited and then allowed a suitable time for equilibration. Samples of solution from the 'unlabelled' side of the chamber are taken and counted, and the fluxes calculated as μequiv./cm^2 per h [20]. The effect of fatty acid applications to one cornea of the pair can then be determined by comparison of the flux data with the untreated cornea.

Fatty acids

Purified fatty acids were obtained from Sigma, and stock solutions prepared in methanol. Unsaturated fatty acid stocks were prepared as 100 mg/ml methanol, and saturated fatty acid stocks either as 25 or 50 mg/ml. The addition of fatty acids to the chamber was either by direct application of μl quantities of methanol stock, with dilution occurring in the chamber, or by premixing of methanol stock with Ringer's solution or Ringer's supplemented with 10% fetal calf serum, followed by brief ultrasonication before addition to the chamber. The latter treatment was found necessary for some of the saturated fatty acids which are difficult to solubilize. Rapid addition of selected fatty acids to either or both corneal surfaces is easily accomplished without disturbing the cornea.

Measurement of plasma membrane lipid fluidity

Preparation of cells. Cell suspensions of rabbit croneal epithelium were prepared by scraping the epithelium. After 0.5 h of incubation in Ca^{2+}- and Mg^{2+}-free Ringer's solution with 0.5% pronase at 37°C, the suspensions were found to consist mainly of single cells. Cell suspensions were centrifuged and the solution replaced with Minimal Essential Medium supplemented with 5% fetal calf serum. Cells were then allowed 1 h for recovery at room temperature. Trypan blue dye exclusion tests were used to indicate viability, and viability was found to be consistently greater than 96%.

Frog corneal epithelial cells were found to be extremely fragile, and therefore required the following modified procedure. Corneas were scraped into Ca^{2+}- and Mg^{2+}-free Ringer's and allowed to dissaggregate for 10 min at room temperature with mild intermittent agitation. Before dissaggregation was com-

plete, cells were briefly centrifuged and the suspending medium replaced with fresh Ringer's. Concentrated cell suspensions were then transferred to 0.5 ml, capacity Sykes-Moore microculture dishes (Bellco), and cells were allowed 1 h to settle and adhere to the lower cover slip. The advantage of this technique is that solutions bathing the cells can be changed without having to resort to repeated centrifugation steps which seem to damage these fragile cells.

Immunofluorescence staining of plasma membrane. Concentrated suspensions of rabbit corneal epithelial cells in phosphate-buffered saline were cooled on ice in preparation for antigenic labelling of plasma membrane proteins. The entire procedure was carried out at 0° C. Goat anti-rabbit antiserum (Cappel) was added to cell suspensions which were then washed and mixed with fluorescein-conjugated rabbit anti-goat immunoglobulin. Cells were labelled with each antibody for 15 min. After a second thorough washing, cell suspensions were observed immediately by fluorescence microscopy, or incubated at 37° C for 15 min to allow patch formation, and then fixed in 2% glutaraldehyde.

Frog corneal epithelial cells were labelled first with anti-frog antiserum, raised in rabbit. The antiserum, diluted 1 : 5 with iced Ringer's was added to precooled Sykes-Moore chambers containing the cells by perfusion through the side gasket, and excess fluid exited via a needle at the opposite side. After incubation for 15 min, the first antibody was washed out by chamber perfusion with 5 ml iced Ringer's followed by the addition of fluorescein-conjugated goat anti-rabbit immunoglobulin (Cappel) as the second label. Following a final washout, the chambers were either observed immediately by fluorescence microscopy, or brought up to room temperature for 15 min to facilitate membrane diffusion and patching, and observed after addition of 2% glutaraldehyde.

Observation and quantitation of membrane fluidity. Fluorescent cells and cell fractions were observed and photographed with an epi-fluorescence equipped Zeiss research microscope.

After a 15 min incubation period during which lateral diffusion of membrane components was allowed to occur, differential cell counts were done to determine the percentage of cells showing fluorescent patches of the membrane as opposed to those showing continuous ring staining. Patching is the visible end result of protein diffusion within the membrane, a parameter determined by membrane lipid viscosity. Therefore, by comparing treated and control cell populations for percentage of cell patching, we can determine changes in membrane fluidity resulting from incorporation of fatty acids into membrane lipids.

Incorporation of [^{14}C]linoleic acid into scraped rabbit corneal epithelium

Corneal epithelial scrapings from four corneas were incubated in Ringer's solution containing [^{14}C]linoleic acid (1 μCi/1 $\cdot$ 10^7 cells) for 20 min at 37° C. Cell membranes were isolated by the technique of Curtis et al. [28]. In summary, incorporation was followed by Dounce homogenization of cell pellets in a sucrose/$MgSO_4$ medium buffered with Tris-HCl at pH 7.4. The homogenate was centrifuged at 5000 $\times g$ for 15 min, and the supernatant was then recentrifuged at 30 000 $\times g$ for 30 min. The pellet, containing the crude membrane fraction, was resuspended in 1 $\cdot$ 10^{-3} M Tris/$MgSO_4$ solution, pH 8.6, and layered over a Ficoll solution of density 1.050 at 20° C. A final centrifugation at 1.3 $\cdot$ 10^5 $\times$ g for 80 min resulted in the appearance of an

opalescent band at the Ficoll-buffer interface, which was recovered as the membrane fraction.

The relative purity of the membrane fraction obtained by this method was determined by incubating intact cells with the above immunofluorescent label and carrying them through the membrane isolation procedure. The resulting membrane fraction yields an intensely bright fluorescence when viewed by fluorescence microscopy, while fluorescence of the nuclear/cytoplasmic fraction was virtually nil, indicating a highly enriched membrane fraction.

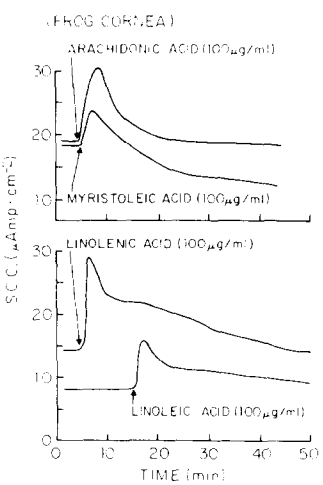
Specificity of antibody

Goat anti-rabbit and rabbit anti-frog antisera were checked for specificity of binding by immunofluorescence labelling of washed rabbit and frog erythrocytes with the above antisera and also with normal goat and normal rabbit sera. Rabbit erythrocytes were found to show strong ring fluorescence when labelled with goat anti-rabbit antiserum, but not with normal goat serum. Likewise, frog erythrocytes showed ring fluorescence when labelled with rabbit anti-frog antiserum, but not with normal rabbit serum, indicating that these antisera are highly species specific.

Results

Ussing chamber experiments

Unsaturated fatty acids. The addition of unsaturated fatty acids at a concentration of 100 μg/ml to transport chambers containing frog or rabbit corneas resulted in every case in a sizeable increase in the short-circuit current, indicating an increase in the rate of active ion transport across the cornea (Figs. 1 and 2). The response in frog cornea was characteristically rapid in



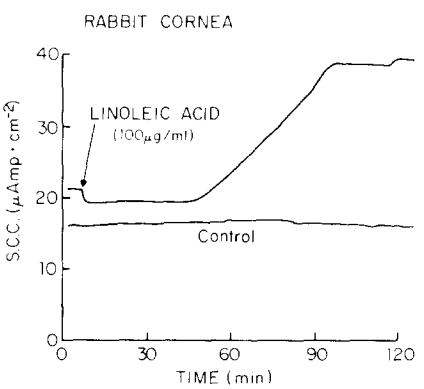


Fig. 1. Stimulation of short-circuit current in isolated frog cornea by unsaturated fatty acid application to both corneal surfaces as indicated by arrows.

Fig. 2. Stimulation of short-circuit current in isolated rabbit cornea by linoleic acid application to both corneal surfaces.

onset, but transient, while in rabbit cornea a delay in onset of the response was observed (Fig. 2). It is likely that the much greater thickness of rabbit cornea compared to that of frog may account for this difference.

Four different unsaturated fatty acids varying in chain length and degree of unsaturation were added to mounted frog corneas. Myristoleic acid (14 : 1) elicited the smallest response: a 28% increase over untreated control corneas. Arachidonic acid (20 : 4) elicited the largest increases in short-circuit current for frog cornea, with an average increase of 65% (see Table I). The largest response observed for one experiment was 115% over control for arachidonic acid.

It is well-known that frog corneas exhibit a rather large variability in their response to various drugs, dependent upon, among other unknowns, seasonal factors and general health of the animal. To minimize the variability, experiments were always done on corneal pairs from the same animal. Nevertheless, the data clearly show that large increases in current can be produced very quickly upon the addition of unsaturated fatty acids. Also, clear differences are shown in the relative effectiveness of different unsaturated fatty acids in stimulating the short-circuit current.

Saturated fatty acids. A series of experiments were done in which three different saturated fatty acids were administered to mounted frog corneas. The fatty acids, palmitic, stearic and arachidic acids (16-, 18-, and 20-C, respectively) were added to the chambers either directly from methanol stock, or premixed with Ringer's and 10% fetal calf serum, with final concentrations of from 16 to 80 μg/ml. These saturated fatty acids did not produce any change in either current or potential. We then took frog corneas which were first treated with

TABLE I

EFFECTS OF VARIOUS FATTY ACIDS ON THE SHORT-CIRCUIT CURRENT (SCC) OF ISOLATED FROG AND RABBIT CORNEAS

Corneas were mounted in Ussing-type chambers as described in the text, and fatty acids were added to the medium bathing both corneal surfaces with a final concentration of 100 μg/ml for the unsaturated fatty acids, and 16—80 μg/ml for the saturated fatty acids. The data are expressed as the mean percentage increase over control levels, ± S.E. Note that for frog cornea, arachidonic acid (the most highly unsaturated fatty acid used) appears to have the greatest ability to increase SCC, while saturated fatty acids have no effect on SCC.

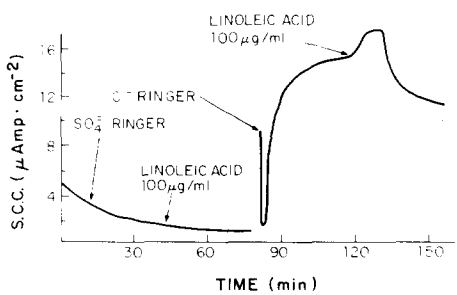
	Unsaturated fatty acids	Chain length: unsaturations	% increase in SCC ± S.E.	No. of expts.
Frog cornea	Myristoleic	(14 : 1)	28 ± 7.7	5
	Linoleic	(18 : 2)	44 ± 8.9	9
	Linolenic	(18 : 3)	45 ± 14.0	5
	Arachidonic	(20 : 4)	65 ± 11.6	7
Rabbit cornea	Linoleic	(18 : 2)	69 ± 9.1	5
	Saturated fatty acids	Chain length: unsaturations	% increase in SCC ± S.E.	No. of expts.
Frog cornea	Palmitic	(16 : 0)	0	5
	Stearic	(18 : 0)	0	8
	Arachidic	(20 : 0)	0	4

saturated fatty acids as above, and added the unsaturated linoleic acid to the treated and control corneas, in order to determine whether saturated fatty acids might antagonize the response to linoleic acid. However, no difference in the short-circuit current increase elicited by linoleic acid between pretreated and control corneas was noted. It therefore appears that the above saturated fatty acids have no effect on frog corneal short-circuit current, and furthermore, do not interfere with the action of unsaturated fatty acids.

Chloride flux. It is well-known that the major ion to be transported by frog cornea is Cl^-, transported from endothelial to epithelial side. The transport mechanism is located in the epithelium, and apparently controls the hydration and transparency of the cornea [20]. To demonstrate that increases in short-circuit current after addition of unsaturated fatty acids are a measure of increased chloride transport across frog cornea, experiments similar to those above were done while corneas were bathed in chloride-free Ringer's solution. In the absence of chloride, consistently low short-circuit currents were obtained and no changes in current could be elicited upon addition of any fatty acids. When, however, the same corneas were returned to normal Ringer's, typical short-circuit currents and responses to unsaturated fatty acids were again observed (Fig. 3).

To further show that increases in short-circuit current indicate activation of chloride transport, $^{36}Cl^-$ efflux measurements were done on six frog corneas. Upon addition of linoleic acid to mounted corneas, the mean active chloride flux was found to increase by 56% (±11.1). The flux data, as well as the results obtained with chloride-free Ringer, indicate that it is the transcorneal active chloride transport that is stimulated by unsaturated fatty acid treatment. Since the short-circuit current and chloride flux undergo simultaneous increases following linoleic acid treatment, we conclude that changes in current indicate similar changes in chloride flux (Fig. 4).

Inhibitors of prostaglandin synthetase. It is well-known that arachidonic acid as well as other unsaturated fatty acids serve as precursors in the synthesis of prostaglandins. It has also been shown that prostaglandins can cause increases



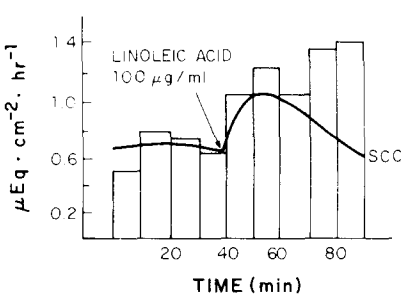


Fig. 3. Isolated frog corneas bathed in chloride-free (sulfate) Ringer's show no response to linoleic acid application. When returned to standard (chloride-containing) Ringer's, the same corneas once again show typical response to linoleic acid treatment. Brief removal of fluid from corneal chambers during the wash out results in a discontinuity in the graph, followed by a rapid return of current to its former level.

Fig. 4. Addition of linoleic acid to mounted frog corneas results in the stimulation of $^{36}Cl^-$ active flux (bars) simultaneously with short-circuit current.

in short-circuit current across mounted frog cornea due to stimulation of chloride transport [25]. In order to test whether prostaglandins may play a role in the fatty acid stimulation of chloride transport, two inhibitors of prostaglandin synthetase, aspirin and indomethacin, were administered to mounted frog corneas to determine whether or not they would affect the observed action of arachidonic and linoleic acids on short-circuit current. At concentrations known to inhibit prostaglandin synthesis in other systems [29], aspirin at 100 μg/ml and indomethacin at 30 μg/ml had no inhibitory effect on the action of arachidonic or linoleic acid. We therefore conclude that de novo prostaglandin synthesis is not required in the fatty acid stimulation of chloride transport (Fig. 5).

Membrane fluidity

Membrane fluidity assays were done on isolated rabbit and frog corneal epithelial cells as described above. Prior to immunofluorescence labelling, cells were incubated in the presence of linoleic acid (200 μg/ml) for 10 min at 37°C for rabbit cells, and room temperature for frog cells. Control cells were not treated with fatty acid.

The basis for the assay lies in the fact that antigenic membrane proteins are free to diffuse laterally within the plane of the membrane at a rate which is limited primarily by membrane lipid viscosity. When fluorescent antibody binds to antigenic membrane proteins they are randomly distributed in the plane of the membrane. Since the immunofluorescence labelling procedure is carried out at 0°C (which elevates viscosity and retards diffusion), the initial distribution of fluorescent label can be observed by fluorescence microscopy as diffuse, homogeneous fluorescent rings (Fig. 6). After labelling, cells are given a 15 min incubation period at normal physiological temperature (37°C for rabbit; room temperature for frog), during which time membrane lipids are at normal viscosity, once again allowing the lateral diffusion of the labelled antigens. Because antigens are bound to polyvalent antibody, any collisions of antigens that occur during diffusion result in cross-linking, and aggregates or clusters begin to form. When these become sufficiently large, they become visible as fluorescent patches when viewed by fluorescence microscopy (Fig. 7).

Differential cell counts on rabbit as well as frog corneal epithelial cells revealed an increase in membrane fluidity as indicated by an increase in fluores-

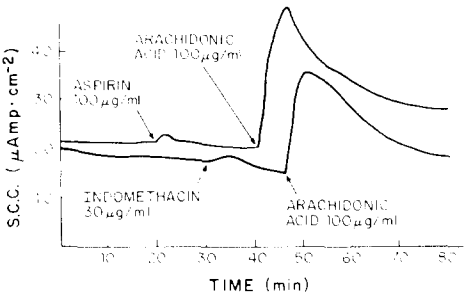


Fig. 5. Two different inhibitors of prostaglandin synthetase (aspirin and indomethacin) have no effect on the typical response of mounted frog corneas to arachidonic acid.

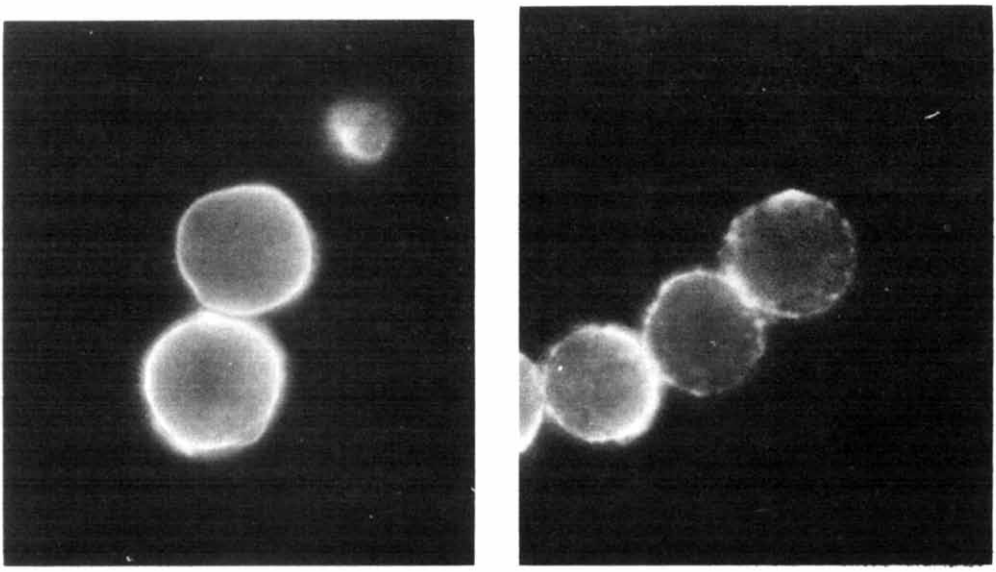


Fig. 6. Isolated frog corneal epithelial cells as seen by fluorescence microscopy immediately following immunofluorescence labelling of the cell surface. Diffuse fluorescent rings are apparent.

Fig. 7. The same cells following immunofluorescent labelling and incubated for 15 min at physiologic temperature. Patching of fluorescence on the cell surface is apparent.

cent patching on the cell membrane of cells exposed to linoleic acid, compared to unexposed controls (Table II).

Fatty acid incorporation

[^{14}C]Linoleic acid incorporation into whole cells after 20 min was 27% of the applied label, equivalent to 1.5 μg of linoleic acid/1 · 10^7 cells. Incorporation into the cell membrane fraction was 49.7% of the total radioactivity taken up by the cells. This corresponds to the fatty acid incorporation data of Curtis et al. [28,38], in which some 2 μg of oleic acid/1 · 10^7 cells were found to incorporate into whole cells under similar conditions. These authors also determined that 70% of membrane incorporation was into phospholipids, and the remainder into neutral lipids. In the same study, incorporation of a large number of different saturated and unsaturated fatty acids into cell membranes was found to be roughly identical.

It has been shown that fatty acid incorporation directly into membrane phospholipids is controlled by specific acyl transferases which show 1- and 2-position specificities [30]. Other cell organelles are apparently not required for incorporation, as isolated membrane preparations have been shown to incorporate fatty acids [28]. The rapid response of frog corneal short-circuit current following additions of unsaturated fatty acids is consistent with the idea of their rapid substitution into the membrane. Whether or not fatty acids are built into the phospholipids through the action of an acyl transferase system is not known, and we do not rule out simple insertion in the lipid bilayer.

TABLE II

MEMBRANE FLUIDITY CHANGES INDUCED BY LINOLEIC ACID TREATMENT OF ISOLATED RABBIT AND FROG CORNEAL EPITHELIAL CELLS

Concentrated cell suspensions were prepared and incubated with linoleic acid (200 μg/ml) for 10 min prior to immunofluorescence labelling of the cell surface. Control cells were not treated with fatty acid. Labelling with fluorescent antibody is done on ice. Cells are then returned to physiological temperature for 15 min to allow for lateral diffusion of labelled membrane antigens. Collisions between polyvalent antibody molecules during diffusion results in cross-linking, producing antibody clusters within the membrane which, when sufficiently large, may be seen as fluorescent patches by fluorescence microscopy. Since the rate of patching is limited primarily by the rate of antibody diffusion in the plane of the membrane, patching may be used as an indicator of membrane viscosity (fluidity). Each cell counted by microscopy is classified as having either ring fluorescence or patching of fluorescence. Results of four such experiments are shown, three of which were done on rabbit, and one on frog corneal epithelial cells. In each experiment the data indicate that linoleic acid treated cells have a higher incidence of patching than untreated control cells.

	Experiment No.	No. cells counted	Rings	Patches	% patches
Rabbit cells					
Linoleic acid	1	67	26	41	61
treated	2	113	37	76	67
	3	199	72	127	64
Control	1	157	114	43	27
	2	85	46	39	46
	3	129	69	60	47
Frog cells					
Linoleic acid treated	4	205	118	87	42
Control	4	210	146	64	30

Discussion

The present experiments have demonstrated that it is possible to alter membrane lipid fluidity in corneal epithelium by a relatively brief incubation with selected fatty acids. Furthermore, the results indicate a relationship between membrane fluidity and chloride transport.

On theoretical grounds, the substitution of unsaturated fatty acids into membrane phospholipids would be expected to increase membrane lipid fluidity. These molecules have a greater degree of motional freedom and lower melting points with increasing unsaturation, and thus can be expected to exert a fluidizing effect when inserted into the bulk membrane. We have shown that a 10 min incubation of corneal cells with linoleic acid is sufficient to induce a substantial increase in membrane lipid fluidity (as reflected by patching of fluorescent antibody). These results are in accordance with expectations based on theory.

In addition, we have found that the unsaturated fatty acids used in these experiments were able to induce a rapid but transient increase in short-circuit current across frog cornea. The reason for the transient nature of this effect is not known, but one possibility is that a rapid homeoviscous adaptation is occurring in while the cells readjust the fatty acid balance and thereby return membrane fluidity to normal. There is some recent evidence in the literature

which supports this. The studies by Doi et al. [39], and Mandel et al. [40] both show that when cells are supplemented with an unsaturated fatty acid (18 : 3) the percent of the saturated (18 : 0) fatty acid in the membrane increases markedly, in addition to the 18 : 3 fatty acid. It is also known that cells are able to desaturate, saturate or alter chain length of incorporated fatty acids through action of various enzymes, and this might account for the short duration of the effect.

Experiments done previously in this laboratory have been concerned with the measurement of Cl^- transport across frog and rabbit corneal epithelium. In the course of these studies many agents have been uncovered which have been found to stimulate Cl^- transport, producing their effects principally through increases in the concentration of cyclic AMP. In fact, cyclic AMP by itself has the ability to stimulate Cl^- transport. Catecholamines, particularly epinephrine, have been shown to produce rapid and sustained stimulation of Cl^- transport as indicated by measurements of short-circuit current across the cornea [23,24]. Other agents known to increase cyclic AMP levels, such as theophylline, ascorbic acid, prostaglandins, etc., have also been found to stimulate Cl^- transport [25,31]. It is thought that corneal epithelial cell membranes have specific receptors for these drugs which have a common action on adenylate cyclase or phosphodiesterase to increase cyclic AMP levels.

According to the mobile receptor hypothesis [4], receptors and the enzymes they regulate diffuse independently within the plane of the cell membrane. When a ligand-receptor complex forms, its affinity for the enzyme becomes greater. At this point, activation of the enzyme depends upon the chance encounter of similar recognition sites on receptor and enzyme. Increasing membrane fluidity should result in a higher lateral diffusion rate, which would increase the chances of receptor interaction with the enzyme. Consistent with this model are numerous observations that membrane fluidization activates enzyme systems within the membrane. Orly and Schramm [8] demonstrated that the insertion of unsaturated fatty acids into the membrane of turkey erythrocytes enhanced hormone activation of adenylate cyclase. In another study, the activity of membrane-bound neuraminidase on ganglioside degradation was found to increase when membrane fluidity (measured by fluorescence depolarization) was increased through use of anesthetics [32]. A number of reactivation studies with lipid-depleted $(Na^+ + K^+)$-ATPase preparations suggest that the amount of reactivation depends on the nature of the fatty acyl groups and membrane fluidity [7,33].

Fatty acid activation of chloride transport in cornea can be interpreted to result from an increase in membrane fluidity which in turn, results in greater interaction between adenylate cyclase and the receptor complex, with increased cyclic AMP production as an end result. Indeed, Burstein et al. [34] have shown that arachidonic acid causes a sharp increase in cyclic AMP levels in primary epithelial cell cultures within 2 min of application. Although their data do not elucidate a mechanism for such an increase, the rapidity of the rise in cyclic AMP seems to lessen the probability that endogenous synthesis of prostaglandin is responsible. In support of this, we have obtained direct evidence of the inability of prostaglandin synthetase inhibitors to suppress the stimulation of chloride transport by fatty acids. Much work remains to be done

in order to demonstrate the validity of the above model, especially with regard to cyclic AMP measurements in our system, and alternative mechanisms cannot currently be ruled out. For example, the fluidization of cell membranes by unsaturated fatty acids may result in conformational changes in transport proteins which allows them to transport ions more efficiently. Another model for active ion transport has been described by Singer [35], in which aggregates of integral protein subunits within the membrane (perhaps tetramers) form pores through which specific ions are transported via a conformational change or rearrangement among the subunits. An increase in membrane fluidity could conceivably promote the increased aggregation of such protein subunits, resulting in a greater number of membrane pores and consequently, the enhancement of active ion transport. That alterations in the distribution of integral proteins within the membrane occurs has been demonstrated by freeze-fracture studies involving intramembraneous particles [36,37].

More experimentation is required to decide which, if any, of the above mechanisms actually applies. However, our data clearly show that unsaturated fatty acids tend to fluidize the cell membrane, permitting a more rapid lateral mobility of intrinsic membrane proteins. The increase in membrane lipid fluidity and the simultaneous increase in active chloride transport appear to be causally related events.

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