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RESEARCH COMMUNICATION

The chloride channel blocker anthracene 9-carboxylate inhibits fatty acid incorporation into phospholipid in cultured human airway epithelial cells

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This study investigated whether making epithelial cell membranes impermeable to Cl^- movement affects incorporation of fatty acids into membrane constituents. Epithelial cells were isolated from human nasal polyps, cultured for 5–7 days, and used to test the effect of anthracene 9-carboxylate (9-AC), known to inhibit Cl^- conductance across the epithelial membrane, on the incorporation and desaturation of [1-^{14}C]linoleic acid ($C_{18:2,n-6}$) in experiments of up to 4 h duration. 9-AC (5 mM) reduced $C_{18:2,n-6}$ incorporation into phospholipid by 60–70%, and increased incorporation of $C_{18:2,n-6}$ into triacylglycerol by 50–100%. The decrease in $C_{18:2,n-6}$ incorporation into phospholipid was rapid and dependent on the concentration of 9-AC. Substitution of extracellular Cl^- with gluconate significantly decreased $C_{18:2,n-6}$ incorporation into phospholipid, suggesting that the effect of 9-AC may occur by inhibiting Cl^- conductance. Lipid analysis of cells exposed to 50 μM-$C_{18:2}$ revealed that, as a consequence of the effect of 9-AC, the level of $C_{18:2,n-6}$ in cell membrane phospholipid was significantly lowered. The relative rate of $C_{18:2,n-6}$ desaturation was not apparently changed by 9-AC. These data suggest that Cl^- conductance may play a role in fatty acid incorporation into epithelial cell membrane phospholipids.

INTRODUCTION

Cystic fibrosis is a lethal inherited disease with a high incidence in the Caucasian population. The primary defect of the disease is the failure of an epithelial cell Cl^- channel to respond to cyclic AMP [1–6]. As a result, the permeability of epithelial tissues to Cl^- is lost or severely reduced. In airway epithelial cells this leads to an imbalance in ion and fluid transport, believed to cause abnormal mucus secretion and ultimately results in pulmonary infection and epithelial cell damage. One of the principal features of the disease also involves low essential fatty acid levels (particularly linoleic acid; $C_{18:2,n-6}$), independent of pancreatic insufficiency and therefore not the result of malnutrition [7–11]. Although the possibility of increased turnover of essential fatty acids has been proposed [12–16], definition of the mechanism of the membrane fatty acid abnormality remains controversial. It is also uncertain if the reduced membrane level of $C_{18:2,n-6}$ is related to the pathophysiology of the disease. Whether or not impermeability of the epithelial cell membrane to Cl^- and other ions affects fatty acid metabolism is also unknown. Therefore the present study was initiated to examine the effect of anthracene 9-carboxyate (9-AC), under conditions known to inhibit Cl^- conductance across epithelial membranes [2,17–21], on the incorporation of $C_{18:2,n-6}$ and palmitic acid ($C_{16:0}$) into the membrane and subsequent desaturation of $C_{18:2,n-6}$ in cultured human airway epithelial cells.

MATERIALS AND METHODS

This research was approved by the Research Ethics Board of the Faculty of Medicine, University of Alberta, Edmonton, Canada.

Materials

The radioactive materials [1-^{14}C]$C_{18:2,n-6}$ (50 mCi/mmol), [1-^{14}C]$C_{18:2,n-9}$ (oleic acid) (57 mCi/mmol) and [1-^{14}C]$C_{16:0}$ (56 mCi/mmol), of more than 90% purity, were purchased from NEN and used without further purification. 9-AC, amiloride, verapamil, sodium gluconate, unlabelled fatty acids [$C_{18:2,n-6}$, $C_{18:3,n-6}$ (linolenic acid), $C_{20:3,n-6}$ (dihomo-γ-linolenic acid) and $C_{20:4,n-6}$ (arachidonic acid)], lipid standards and other biochemicals were obtained from Sigma (St. Louis, MO, U.S.A.). All solvents were redistilled before use.

Cell isolation and culture

Primary cultures of epithelial cells from human nasal turbinates were obtained by methods similar to those described by Yankaskas *et al.* [22]. Turbinates, received 1–4 h after surgery, were placed in Joklik's modified minimum essential medium (MEM) supplemented with antibiotics (100 units of penicillin G/ml, 100 μg of streptomycin sulphate/ml and 50 μg of gentamycin/ml) at 4 °C for transport from surgery. Tissues were transferred to a solution of 0.1% (w/v) type 14 proteinase in MEM with the same antibiotics and digested overnight at 4 °C with mild agitation. After raising the temperature to 37 °C for 1–2 h in a 5% CO_2 incubator, the cell suspension was collected and 10% (v/v) fetal bovine serum (FBS) was added to neutralize the proteinase. The cells were filtered through a 60 μm Nitex mesh, centrifuged at 275 *g* for 10 min and washed once in Dulbecco's modified Eagle's medium (DME)/Ham's F_{12} containing 10% FBS. The resulting cells were suspended in culture medium (DME/F_{12} + hormones + antibiotics + 1% FBS; this supplemented medium was similar to that described by Yankaskas *et al.* [22]) and plated on collagen-coated plates, and then cultured

Abbreviations used: 9-AC, anthracene 9-carboxylate; MEM, minimum essential medium; FBS, fetal bovine serum; DME, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulphoxide; DPC, diphenylamine 2-carboxylate; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid.

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at 37 °C in 5% CO_2 at 98% relative humidity in a tissue culture incubator (Model 3173; Forma Scientific). The culture medium was changed every other day. After 5–7 days in culture, cells had grown to confluence and were used for biochemical assay.

Isotope incubation

Confluent cells were detached from the plate surface with 0.25% (w/v) trypsin solution. Cells were collected in a centrifuge tube, neutralized with 10% FBS, washed once with culture medium and suspended in fresh culture medium (without FBS) and counted in a haemocytometer [(3–5) × 10^6 epithelial cells/culture, with a purity of more than 90%]. An aliquot was removed for analysis of protein, with BSA as standard [23]. The remaining cells were replated on 60 mm-diam. plastic culture dishes at a density of (5–8) × 10^5 cells in 2 ml of medium. Mixtures of 1-^{14}C-labelled fatty acids and unlabelled fatty acids, suspended by sonication at 37 °C in sterile 5% (w/v) BSA [24], were added in a 100 μl volume to give a final fatty acid concentration of 10 μM plus 2.2 × 10^6 d.p.m. of ^{14}C-labelled fatty acid (2.2 × 10^4 d.p.m./nmol of fatty acid, unless otherwise specified), or 40 μM for unlabelled fatty acids. Cells were incubated for various lengths of time under the conditions described above.

To test the effects of ion channel blockers, chemicals (9-AC, amiloride, verapamil, sodium gluconate) were added to the cells 5 min before addition of labelled fatty acids. 9-AC (5 mM, unless otherwise specified) was dissolved in 10 μl of dimethyl sulphoxide (DMSO) at a final concentration of 0.5% (w/v). At this concentration, DMSO alone did not interfere with fatty acid metabolism.

For experiments using Cl^--free solutions, culture medium was replaced by a buffer solution containing 1.2 mM-$CaSO_4$, 1.2 mM-$MgSO_4$, 118.9 mM-sodium gluconate (NaCl for control), 20.4 mM-$NaHCO_3$, 2.4 mM-K_2HPO_4 and 0.6 mM-KH_2PO_4. In this solution, the cells were incubated with fatty acids for only 2 h.

Lipid extraction and analysis

After incubation, the cells were harvested with a rubber policeman in the presence of 1 ml of 0.25% trypsin (if necessary), and culture medium was removed by centrifugation. Cell pellets were washed twice with phosphate-buffered saline (KCl, 0.2 g/l; KH_2PO_4, 0.2 g/l; NaCl, 8 g/l; Na_2HPO_4, 1.15 g/l, pH 7.4). Cell pellets and culture medium plus wash buffer were extracted with chloroform/methanol (2:1, v/v) [25] containing 0.005% butylated hydroxytoluene as antioxidant. Total phospholipid and triacylglycerol were separated by t.l.c. on silica gel G plates using a solvent system comprising light petroleum (b.p. 38–58)/diethyl ether/acetic acid (80:20:1, by vol.) [26]. Fatty acids were methylated with 14% (w/w) BF_3/methanol reagent and heated for 1 h at 100 °C [27]. Fatty acid methyl esters were separated according to degree of unsaturation on silica gel t.l.c. plates impregnated with $AgNO_3$ [final concentration of 30% (w/w) $AgNO_3$ in silica] using hexane/diethyl ether/acetic acid (94:4:2, by vol.) for 35 min and then the same solvents (90:10:2, by vol.) as sequential solvent systems for 25 min. Bands containing dienoic, trienoic and tetraenoic fatty acids could be identified by comparison with reference standards.

Analysis of phospholipid fatty acid composition was carried out by g.l.c. using a fully automated Varian Vista 6000 GLC equipped with a flame-ionization detector [28]. These analytical conditions separate all saturated, mono-, di- and poly-unsaturated fatty acids from C_{14} to C_{25} chain length. A Varian Vista 402 data system was used to analyse percentage area for all resolved peaks.

Liquid scintillation counting

All labelled samples separated by t.l.c. were scraped directly from plates into scintillation vials containing 5 ml of scintillation cocktail (Aquasol; NEN). Samples were counted for radioactivity in a Beckman LS-5801 liquid scintillation counter. Counting efficiency was determined to be more than 95%.

Statistical analysis

Results are presented as means ± S.D. The significance of the effects of 9-AC was determined by analysis of variance procedures [29].

RESULTS

Effect of 9-AC on [1-^{14}C]$C_{18:2,n-6}$ incorporation

To determine if 9-AC alters [1-^{14}C]$C_{18:2,n-6}$ incorporation into cellular lipids, 5 mM-9-AC dissolved in DMSO was added to the culture medium. After 4 h of incubation, lipid analysis revealed that label incorporation into phospholipid was decreased by 60–70% (17.9 ± 3.5 to 5.5 ± 2.1 nmol/mg of protein, $n = 5$), whereas label incorporation into triacylglycerol had increased by 50–100% (7.5 ± 2.8 to 12.3 ± 4.0 nmol/mg of protein, $n = 5$). To determine if DMSO produced effects similar to those observed with 9-AC, DMSO alone was added and found not to alter $C_{18:2,n-6}$ incorporation. Quantification of total phospholipid indicated no difference between control and 9-AC-treated cultures, suggesting that low levels of $C_{18:2,n-6}$ observed after treatment of cells with 9-AC are not due to loss of membrane phospholipid, but result from inhibition of $C_{18:2,n-6}$ incorporation into phospholipid. Separation of total phospholipid showed that the inhibition of $C_{18:2}$ incorporation into phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol by 5 mM-9-AC was approx. 70%, 62% and 60% respectively. The relative conversion rate, based on the ratio of total products formed from $C_{18:2,n-6}$ (e.g. $C_{18:3,n-3}$, $C_{20:4,n-6}$) to total substrate ($C_{18:2}$) taken up by the cells (with or without 9-AC), was not apparently changed by the addition of 9-AC.

The inhibition of $C_{18:2,n-6}$ incorporation into phospholipid by 9-AC was rapid (Fig. 1) and concentration-dependent (Fig. 2). The inhibition had reached a stable state by approx. 30 min;

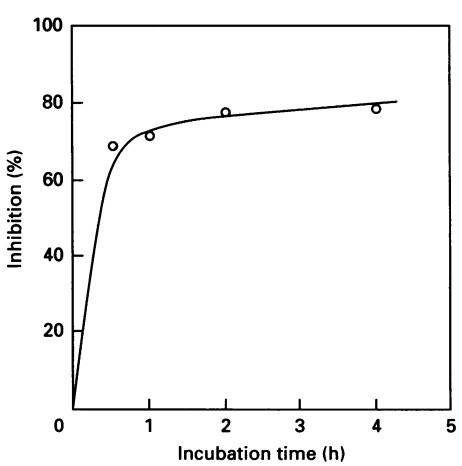

Fig. 1. Time course of the effect of 9-AC on [1-^{14}C]$C_{18:2,n-6}$ incorporation into phospholipid

Cultured epithelial cells were incubated with [1-^{14}C]$C_{18:2,n-6}$ (1 μCi) with or without 9-AC (10 mM) for the time periods indicated. Lipids were extracted and separated on t.l.c., and radioactivity of phospholipids was counted. Results are expressed as percentage inhibition calculated from counting of radioactivity in control and 9-AC-treated samples. Values represent the means of observations with cells from two individuals.

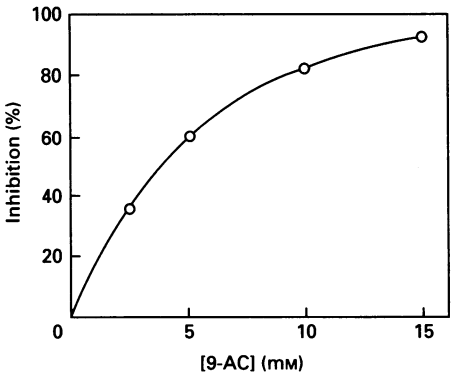

Fig. 2. Effect of 9-AC concentration on [1-^{14}C]$C_{18:2,n-6}$ incorporation into phospholipid

9-AC was added to the culture medium to produce final concentrations as indicated. Cells were incubated with [1-^{14}C]$C_{18:2,n-6}$ for 4 h. Lipids were extracted and separated on t.l.c., and radioactivity of phospholipids was counted. Results are expressed as percentage inhibition compared with the control. Values are the means of observations with cells from two individuals.

Table 1. Effect of ion substitution on the response to 9-AC

Cultured epithelial cells were incubated with [1-^{14}C]$C_{18:2,n-6}$ in either NaCl buffer solution (mM: 1.2 $CaCl_2$, 118.9 NaCl, 20.4 $NaHCO_3$, 2.4 K_2HPO_4 and 0.6 KH_2PO_4) or Cl^--free buffer solution (mM: 1.2 $CaSO_4$, 1.2 $MgSO_4$, 118.9 sodium gluconate, 20.4 $NaHCO_3$, 2.4 K_2HPO_4 and 0.6 KH_2PO_4) containing 9-AC (5 mM) or not, for 2 h. Lipids were extracted and separated on t.l.c. The radioactivity of phospholipid and triacylglycerol was counted. Results are expressed as means ± S.D. of observations from cells of three individuals. Significance of differences: * 9-AC-treated versus control ($P < 0.05$); † Cl^--free versus NaCl ($P < 0.05$).

	[1-^{14}C]$C_{18:2,n-6}$ incorporation (nmol/mg of protein)			
	Into phospholipid		Into triacylglycerol	
Buffer solution	Control	9-AC	Control	9-AC
NaCl	9.2±0.3	4.3±0.3*	3.0±0.3	5.0±0.4*
Cl^--free	5.2±0.6†	4.0±0.5	3.1±0.3	3.6±0.2

5 mM-9-AC decreased $C_{18:2,n-6}$ incorporation into phospholipid by 60%. We also tested the effect of another Cl^- channel blocker, diphenylamine 2-carboxylate (DPC), on $C_{18:2,n-6}$ incorporation. Our preliminary data indicated that 2 mM-DPC decreased $C_{18:2}$ incorporation into phospholipid by approx. 60% (17.2±0.2 to 7.0±0.2 nmol/mg of protein; $n = 3$) and increased $C_{18:2}$ incorporation into triacylglycerol by 64% (6.5±0.7 to 11.0±0.6 nmol/mg of protein; $n = 3$).

Cl^- specificity of the effect of 9-AC

To examine the Cl^- specificity of the inhibition of $C_{18:2,n-6}$ incorporation into phospholipid, epithelial cells were incubated with NaCl buffer and Cl^--free buffer (gluconate substitution). Radiolabel incorporation into phospholipid in Cl^--free buffer was much lower than in NaCl buffer (Table 1). These results suggest that the effect of 9-AC on the incorporation of fatty acid into phospholipid may be via an inhibition of Cl^- conductance.

Table 2. Effect of 9-AC on fatty acid composition of phospholipids

Cells were incubated with 50 μM-$C_{18:2,n-6}$ in medium containing no or 10 mM-9-AC for 4 h. Lipids were extracted, separated on t.l.c. and methylated with BF_3. Fatty acid methyl esters were separated and quantified by g.l.c. ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; $\Sigma n-6$, total $n-6$ fatty acids; $\Sigma n-3$, total $n-3$ fatty acids. Values are means ± S.D. ($n = 3$). Significance of differences versus control: * $P < 0.05$; ** $P < 0.01$.

	Composition (%, w/w)	
Fatty acid	Control	9-AC
$C_{14:0}$	0.8±0.3	0.5±0.2
$C_{15:0}$	1.9±0.2	2.1±0.3
$C_{16:0}$	16.1±1.2	17.3±1.5
$C_{16:1}$	6.1±0.3	5.7±0.3
$C_{18:0}$	11.3±0.2	12.0±0.2*
$C_{18:1,n-9}$	34.0±3.5	37.0±3.1*
$C_{18:2,n-6}$	10.9±0.7	5.5±0.2**
$C_{18:3,n-6}$	0.2±0.1	–
$C_{18:3,n-3}$	0.3±0.1	0.3±0.1
$C_{20:0}$	0.5±0.2	0.6±0.1
$C_{20:1,n-9}$	–	0.7±0.2*
$C_{20:3,n-9}$	0.2±0.1	0.3±0.1
$C_{20:3,n-6}$	4.2±1.5	4.0±1.8
$C_{20:4,n-6}$	6.1±0.3	6.2±0.3
$C_{20:3,n-3}$	0.2±0.1	0.3±0.1
$C_{22:5,n-3}$	1.0±0.2	0.9±0.1
$C_{22:6,n-6}$	1.9±0.6	1.9±0.5
ΣSFA	29.4±2.1	31.5±3.0
ΣMUFA	43.9±3.5	47.3±4.1
$\Sigma n-6$	23.5±1.2	18.1±1.1**
$\Sigma n-3$	3.3±0.5	3.1±0.5

Furthermore, to determine if other ion channel blockers produced effects similar to those observed with 9-AC, the effects of amiloride (a Na^+-channel blocker) and verapamil (a Ca^{2+}-channel blocker) on [1-^{14}C]$C_{18:2,n-6}$ incorporation were tested under identical conditions to those used for 9-AC. There were no differences in radiolabel incorporation into cellular lipids observed between cells incubated with these other ion channel blockers and controls. Label incorporation into phospholipids was 17±0.6, 18±0.5 and 16.8±0.7 nmol/mg of protein for controls and cells incubated with amiloride and verapamil respectively. Label incorporation into triacylglycerol was 8.4±1.0, 9.0±0.6 and 8.3±5 nmol/mg of protein for controls and cells incubated with amiloride and verapamil respectively. These results further suggest that the effect of 9-AC on $C_{18:2,n-6}$ incorporation into cellular lipids is specific to Cl^- permeability.

Effect of 9-AC on incorporation of non-essential fatty acids

To test for a difference between the effects of 9-AC on essential versus non-essential fatty acids, incorporation of [1-^{14}C]$C_{18:1,n-9}$, [1-^{14}C]$C_{16:0}$ and [1-^{14}C]$C_{18:2,n-6}$ into cellular lipids was determined in cells incubated with and without 9-AC. The decreases in incorporation of [1-^{14}C]$C_{18:2,n-6}$, [1-^{14}C]$C_{18:1}$ and [1-^{14}C]$C_{16:0}$ into phospholipid caused by 9-AC were 63±2.4%, 56±2.1% and 38±4.2% respectively ($n = 3$). The increases in [1-^{14}C]$C_{18:2,n-6}$, [1-^{14}C]$C_{18:1}$ and [1-^{14}C]$C_{16:0}$ incorporation into triacylglycerol resulting from 9-AC treatment were 56±5.2%, 100±7.5% and 142±9.6% respectively ($n = 3$). It appears that incorporation of $C_{18:2}$ into phospholipid was inhibited more by 9-AC than was the incorporation of $C_{18:1}$ or $C_{16:0}$. However,

whether this inhibition is specific to polyunsaturated fatty acids requires further experimentation with additional fatty acids.

Effect of 9-AC on fatty acid composition of lipids

Following a 4 h incubation with 50 μM-$C_{18:2,n-6}$, with or without 0.01 mM-9-AC, cellular lipids were extracted and their fatty acid composition was analysed by g.l.c. Cells incubated with 9-AC exhibited a significantly lower level of $C_{18:2,n-6}$ ($P < 0.01$), but higher levels of $C_{18:1,n-9}$ and $C_{20:1,n-9}$ (Table 2). This pattern is similar to that found for cystic fibrosis in previous studies [7–10].

DISCUSSION

The present study demonstrates that 9-AC inhibits incorporation of fatty acids into phospholipids of cultured human airway epithelial cells.

An effect of 9-AC on Cl^- conductance in epithelium has been well documented in previous studies [2,17–21]. 9-AC inhibits Cl^- secretion in airway epithelium by blocking an electrically conductive Cl^- exit step in the apical cell membrane. The inhibition is initially rapid, reaching a stable value by 10–20 min, and is concentration-dependent. 9-AC (4 mM) decreased the single-channel conductance of Cl^- channels in airway epithelium to 68% of control [2], and 6 mM-9-AC inhibited Cl^- secretion by 50% in the intact epithelium [17]. The present study shows a pattern of effect of 9-AC on fatty acid incorporation similar to its known effects on Cl^- conductance (Figs. 1 and 2), indicating a relationship between the blocking of Cl^- conductance across the cell membrane and inhibition of fatty acid incorporation into membrane phospholipids. Further evidence for this relationship can be obtained from the ion substitution studies (Table 1). In the presence of Cl^-, 5 mM-9-AC inhibited $C_{18:2,n-6}$ incorporation into phospholipid by 60%. In the Cl^--free solution the total label incorporated into phospholipid was dramatically reduced, and no significant effect of 9-AC was found. These results suggest that the effect of 9-AC on fatty acid incorporation into phospholipid involves Cl^- conductance or Cl^- concentration.

Recently a number of studies have demonstrated that polyunsaturated fatty acids, particularly arachidonic acid, directly block the Cl^- channel in airway epithelial cells, independent of changes in membrane fluidity or enzymic pathways [30–32]. Our previous findings with airway epithelial cells illustrated that $C_{20:4,n-6}$ inhibits $C_{18:2,n-6}$ incorporation into phospholipid, but increases $C_{18:2,n-6}$ incorporation into triacylglycerol, analogous to the effects of 9-AC (J. X. Kang, S. F. P. Man, N. E. Brown, P. A. Labrecque, M. L. Garg & M. T. Clandinin, unpublished work). Together, these results lead us to speculate that both 9-AC and $C_{20:4,n-6}$ may affect fatty acid incorporation into phospholipids at least in part by inhibition of Cl^- conductance.

Based on our finding that the total incorporation of labelled fatty acids into cellular lipids (phospholipids + neutral lipids) was decreased by 9-AC, the mechanism involved may be an effect on the transport of the fatty acid across the plasma membrane. However, the relationship between inhibition of Cl^- conductance and reduced fatty acid transport across the cell membrane is not known. We suggest three possibilities. First, the impermeability of the membrane to Cl^- results in changes in the electrophysiological properties of the whole cell, thereby directly affecting the functions of cellular protein. It is possible that most whole-cell currents are carried by Cl^- ions. Thus inhibition of Cl^- conductance could produce a dramatic change in electrical potential across cell membranes [17–19]. This may explain why Ca^{2+}- and Na^+-channel blockers did not induce effects on fatty acid incorporation similar to those of 9-AC. Second, inhibition of Cl^- transport may change the intracellular Cl^- concentration, altering the intracellular pH and/or cell volume, with subsequent effects on fatty acid incorporation. Cl^- channels have been shown to be involved in volume regulation in epithelial cells [33], suggesting that membranes can be mechanically modified by Cl^- conductance to influence the functions of membrane proteins. Third, both possibilities noted above may co-exist. However, it may be that the effect of 9-AC is not due to inhibition of Cl^- transport but is caused by competition with fatty acid for incorporation into phospholipid. This appears less likely, because the precondition for 9-AC incorporation is formation of 9-AC-CoA, in which acyl-CoA synthase must have higher affinity for 9-AC than for $C_{18:2,n-6}$, and in this case 9-AC should inhibit $C_{18:2,n-6}$ incorporation into both phospholipid and triacylglycerol. However, the high level of $C_{18:2}$ incorporation into triacylglycerol and the fatty acid profile observed do not support the possible competition of 9-AC with fatty acid.

DPC and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) have been reported to be more potent Cl^--channel blockers in airway epithelial cells [20]. Our preliminary observations show that DPC has a similar effect on $C_{18:2,n-6}$ incorporation to 9-AC, but is more potent. We have not tested the effect of NPPB on fatty acid incorporation because of problems of availability. Whether NPPB can produce a more potent effect on fatty acid incorporation needs further study.

It has been suggested that low levels of $C_{18:2,n-6}$ found in the membrane phospholipids of cystic fibrosis patients are caused by defective fatty acid metabolism [7–9,12–16]. Although many studies have been concerned with the possibility of a defect in fatty acid metabolism in cystic fibrosis, little attention has been paid to mechanisms altering fatty acid incorporation. Active incorporation of fatty acids into the phospholipid of cellular membranes plays a major role in the continuous turnover of membrane phospholipids. Thus it is possible that a defect in incorporation of fatty acids could be partly responsible for the abnormal composition of epithelial membrane phospholipids observed in cystic fibrosis patients. In the present study, since $C_{18:2,n-6}$ incorporation into phospholipid was inhibited by 9-AC, the effect of 9-AC on $C_{18:2,n-6}$ was somewhat greater than that on $C_{18:1}$ and $C_{16:0}$, and the 9-AC-induced change in the fatty acid profile (Table 2) was characterized by a low level of $C_{18:2,n-6}$, it is thus logical to suggest that low levels of $C_{18:2,n-6}$ found in phospholipid in cystic fibrosis can be attributed in part to decreased incorporation of the fatty acid resulting from a defect in Cl^- conductance.

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