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The effect of long-chain *N*-acylethanolamines on some membrane-associated functions of neuroblastoma C1300 N18 cells

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As reported earlier (Gulaya, N.M., Vaskovsky, V.E., Vysotsky, M.V., Volkov, G.L., Govseeva, N.N. and Artemenko, I.P. (1988) Ukr. Biochim. J. 60, 58–63), *N*-acylphosphatidylethanolamines (NAPE) and products of their catabolism, *N*-acylethanolamines (NAE), are present in the lipids of neuroblastoma C1300 N18 undifferentiated cells. The present paper describes the distribution of NAE added to culture medium of differentiated cells and its effect on the fast sodium channels and some other membrane characteristics. It is shown that NAE inhibits the destroying action of veratridine on membranes.

Introduction

Numerous membrane functions are dependent on the lipid composition of the membrane. The activity of membrane-associated enzymes and the transport of cations and metabolites across membranes are affected by their lipid environment. Cell lines maintained in long-term cultivation, such as neuroblastoma cells, provide a valuable tool for the investigation of membrane-related properties. The neuroblastoma C1300 N18 cell line is well studied with well-known properties [1]. Numerous publications deal with the lipid composition of neuroblastoma cells [2–5]. Recently we found two phospholipids with high chromatographic mobility in the phospholipid fraction of neuroblastoma C1300 N18 cells [6]. One of them was identified as *N*-acylphosphatidylethanolamine (NAPE). In recent years this unusual lipid was studied by H.H.O. Schmid and coworkers, who had shown that infarcted areas of canine myocardium accumulated both NAPE [7] and its derivatives *N*-acylethanolamines (NAE) [8]. They had found the biosynthetic link between those two lipids [9,10]. Schmid et al. indicated a high degree of physiological activity of NAE, particularly, that NAE increased the stability of biological membranes. NAE was shown to inhibit permeability-dependent mitochondrial swelling and Ca^{2+} release [11]. Epps et al. [12] discovered that NAE changed Ca^{2+} utilization and Ca^{2+} ATPase in isolated sarcoplasmic reticulum of fast skeletal rabbit muscles. A cytotoxic effect of NAE on several types of neoplastic cells was also shown [13]. Some other biological and physiological properties of NAE were established in experiments in vivo [14,15]. All of these data are summarized in a review by Schmid et al. [16]. Some of the effects of NAE on the cell are thought to occur in the plasma membrane. Epps and Cardin [17] suggested the existence of interaction between NAE and biological membranes. However, the mechanism of NAE action on membranes is unknown.

We have found that the NAPE and NAE content in neuroblastoma cells changed significantly during cell differentiation. NAE was present mainly in plasma membranes of undifferentiated cells. Perhaps this fact has physiological significance [18]. It is known that the excitable plasma membrane of differentiated cells contains a great number of potential-dependent veratridine-activated sodium channels, in contrast to the

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plasma membrane of undifferentiated cells where all these channels are inactive [19]. One can assume that NAE which is located only in the plasma membrane of immature cells can block cation transport through sodium channels. Thus, we decided to investigate the ability of NAE to modify cation transport in neuroblastoma cells.

In the present paper evidence is provided for the effect of NAE on cation transport through veratridine-activated potential-dependent sodium channels and on the basal permeability of plasma membranes of neuroblastoma cells. Veratridine affects not only the function of fast sodium channels, but changes neuroblastoma cell lipid composition as well [20]. Particularly, some of our preliminary results showed that veratridine caused the appearance of lysophospholipids in cell membrane fractions. We studied the influence of NAE on these processes.

Materials and Methods

N-[1-^{14}C]Palmitoylethanolamine. [1-^{14}C]Palmitic acid with spec. act. 5 GBq/l (The State Institute of Applied Chemistry, St. Petersburg, Russia) was used for synthesis of *N*-[1-^{14}C]palmitoylethanolamine.

Cell culture. Differentiated neuroblastoma cells C1300 N18 were used in all experiments. They were cultured in Eagle's medium at 37°C in the presence of 10% bovine serum. To induce neuroblastoma cell differentiation 5′-Br-deoxyuridine (Serva) at a final concentration 40 μmol/l was added to the culture medium. For analysis cells were harvested by Versen solution after 96 h of cell cultivation [19].

Subcellular fractions of neuroblastoma cells. Three subcellular fractions of neuroblastoma cells were prepared by the method of differential centrifugation on Ficoll-400 (Pharmacia, Sweden) [21,22]. Cells were washed 3-times by serum-free Eagle's medium. Cells were sonicated during 3 min at Ultrason (Amemasse, France) at 22 kHz in 10 mol of medium containing 0.5 mM $MgCl_2$, 1 mM dithiothreitol (Serva), 30 mM NaCl, 5 mM PMSF (Serva), 0.02% NaN_3 (Serva), 5 μg/l DNAase (Serva). Then, they were centrifugated 15 min at $2000 \times g$. Supernatant was layered on Ficoll-400 with gradient of density 1.035, 1.065 and 1.075. Three fractions were collected after centrifugation during 60 min at $90\,000 \times g$. The fraction of cytosolic proteins was located in the highest zone (1.035), plasma membranes were harvested in the upper part of the zone with a density of 1.065 and the microsomal fraction was at the bottom of tubes in the 1.075 zone. The volume of each fraction was measured. The protein in these fractions was determined according to Lowry et al. [23] and total radioactivity was counted. The purity of the fractions was assayed as described [21,22].

Assay methods. The function of the potential-dependent sodium channel was studied by the method of Palfrey and Littauer [19]. To load the cells with $^{86}Rb^+$ they were incubated in the serum-free Eagle's medium in the presence of $^{86}Rb^+$ (3–$5 \cdot 10^6$ dpm) for 75 min. Then the radioactive culture medium was discarded, cells were washed 3-times with 3 ml serum-free medium and a new portion of Eagle's medium was added. 640 μg/ml veratridine (Sigma) was added. Aliquots were withdrawn at 3-min intervals for up to 33 min. The scintillation counter SL-4000 (Intertechnique, France) was used for radioactivity measurements. To study the effect of NAE on cation transport it was added in ethanol at final concentrations of $5 \cdot 10^{-7}$, $5 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ mol/l to the culture medium of cells loaded with $^{86}Rb^+$. The final concentration of ethanol was not higher than 0.2%. Afterwards, the radioactivity of aliquots was determined. The remaining part of $^{86}Rb^+$ was determined after cell lysis.

The rate of $^{86}Rb^+$ efflux was determined as the ratio of $\ln[(R_o - R_n)/R_o]$, where R_o is the initial concentration of $^{86}Rb^+$ in the cell and R_n is the quantity of Rb^+ efflux in each interval of time.

Kinetic characteristics of Rb^+ efflux from the cells were determined by the method of Kursky et al. [24]. Five different concentrations of $^{86}Rb^+$ were used: $4 \cdot 10^6$, $5 \cdot 10^6$, $6 \cdot 10^6$, $7 \cdot 10^6$ and $8 \cdot 10^6$ dpm were added to the culture medium. The cells accumulated 0.08, 0.09, 0.11, 0.14 and 0.19 μmol of $^{86}Rb^+$, respectively, per $3 \cdot 10^6$ cells.

Lipid analysis. Lipid extracts were prepared from cell homogenates by the method of Bligh and Dyer [25]. Phospholipids were analyzed by two-dimensional high-resolution micro thin-layer chromatography (HRMTLC) on silica gel [26] by using chloroform/methanol/benzene/ammonium (28%) (65:30:10:6, v/v) and chloroform/methanol/benzene/acetone/acetic acid/water (70:30:10:4:5:1, v/v) [27] as solvent systems.

To determine small amounts of phospholipids molybdate and malachite green spray reagents were used [28,29].

Cholesterol, cholesterol esters, fatty acids and acylglycerols were analyzed by HRMTLC on silica gel in the solvent system hexane/diethyl ether/acetic acid (85:15:1, v/v). Lipids were quantitatively determined by GC on Chrom-5 (ČSSR) with dual flow system FID. A 0.5 m × 3 mm column with 1.5% OV-1 on Shimalite 80–100 mesh (Shimadzu) was used. The temperature program was 100–350°C with a rate increasing 2 C°/min. The flow rate of the carrier gas was 100 ml/min.

3-Fluoro-cholest-5-en (Sigma) trilaurate was used as internal standard. Prior to lipid extraction known amounts of these compounds were added to the cells.

Determination of N-[1-^{14}C]palmitoylethanolamine

and its methabolites in subcellular fractions of cells. Cells were incubated in the presence of $1 \cdot 10^{-5}$ mol/l *N*-[1-^{14}C]palmitoylethanolamine which was synthesized as described by Natarajan et al. [30]. Then they were harvested and subcellular fractions were prepared. Lipid extracts from subcellular fractions were analyzed by HRMTLC on silica gel in the solvent system hexane/diethyl ether/glacial acetic acid (85 : 15 : 1, v/v) in the presence of lipid standards. Zones of silica gel corresponding to standards were dissolved in HF and replaced on scintillation vials.

To determine the quantity of [1-^{14}C]NAE in the lipid extract, HRMTLC with hexane/diethyl ether/acetone/glacial acetic acid (30 : 40 : 20 : 1, v/v) was used as a solvent system in the presence of standard (NAE-16 : 0).

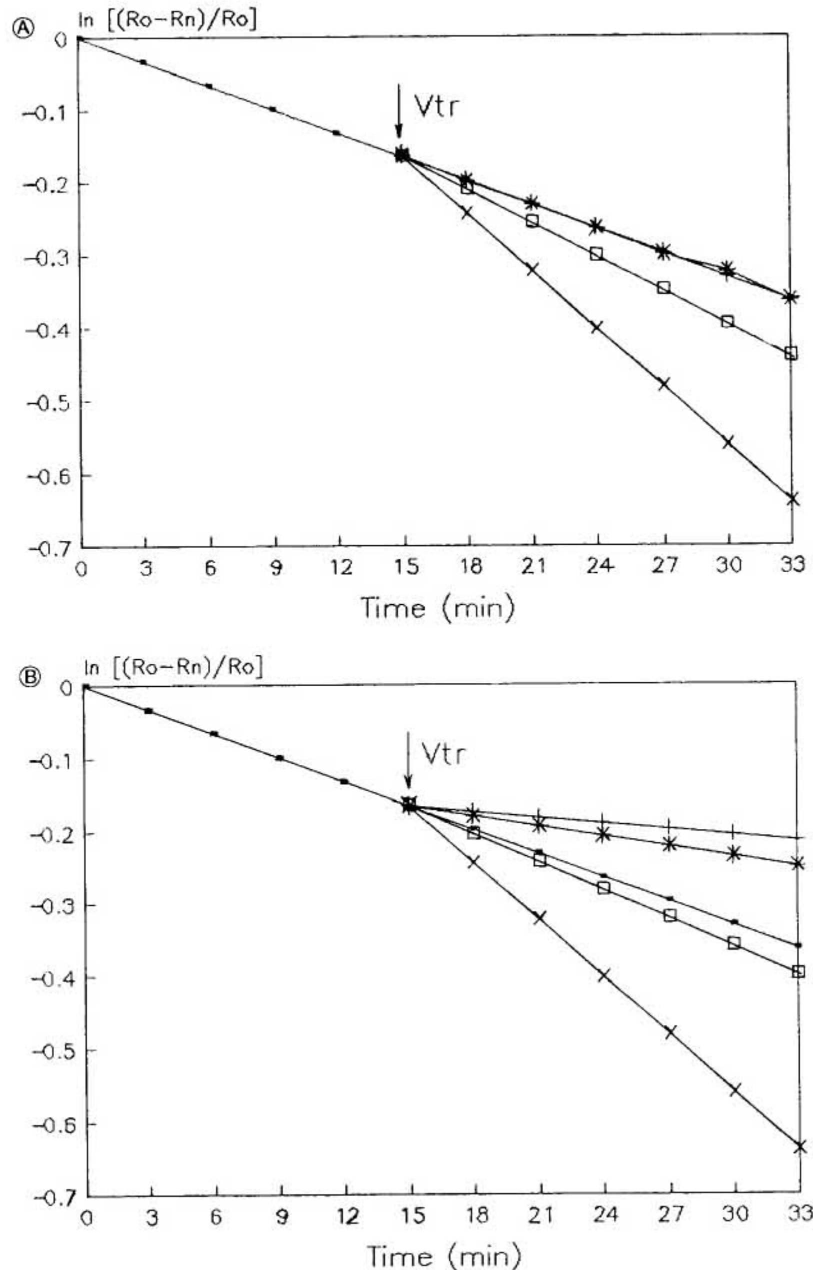

Fig. 1. The influence of NAE-14 : 0 (*A*), NAE-16 : 0 (*B*) and NAE-18 : 0 (*C*) on $^{86}Rb^{+}$ efflux through veratridine-activated sodium channels of differentiated neuroblastoma cells. Basal $^{86}Rb^{+}$ efflux (•); $^{86}Rb^{+}$ efflux in the presence of veratridine (640 μg/ml) (×); $^{86}Rb^{+}$ efflux in presence of veratridine and NAE ($5 \cdot 10^{-5}$ mol/l) (+); $^{86}Rb^{+}$ efflux in presence of veratridine and NAE ($5 \cdot 10^{-6}$ mol/l) (∗); $^{86}Rb^{+}$ efflux in presence of veratridine and NAE ($5 \cdot 10^{-7}$ mol/l) (□). Cells were preloaded with $^{86}Rb^{+}$ for 75 min, radioactive culture medium was discarded, and cells were washed with a new portion of medium. Aliquots of medium were withdrawn at indicated intervals and radioactivity was estimated. Veratridine and NAE were added to the cell culture at the time indicated by the arrow. The rate of $^{86}Rb^{+}$ efflux was determined as $\ln[(R_o - R_n)/R_o]$, where R_o is the initial concentration in the cell and R_n is the value of $^{86}Rb^{+}$ efflux at each interval of time.

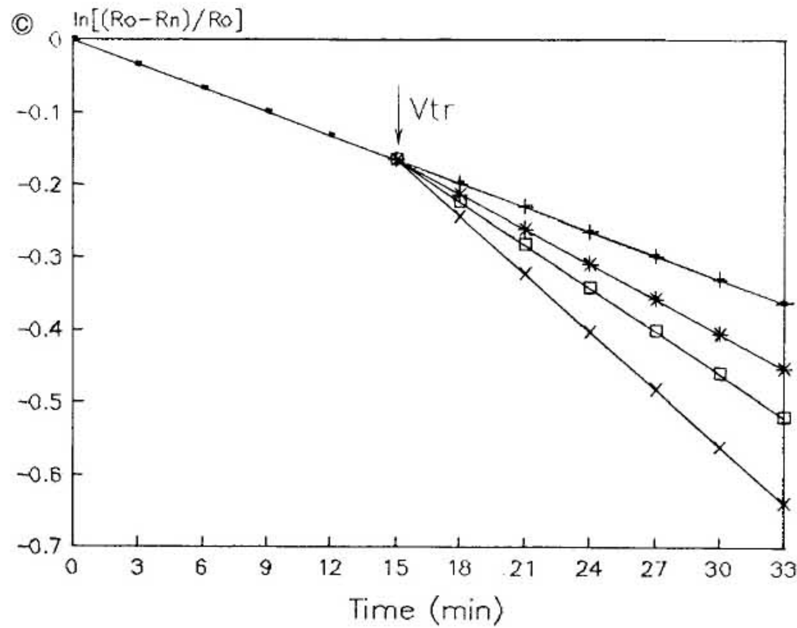

Fig. 1. (continued).

Results

Effect of NAE on cation transport

The effect of NAE on $^{86}Rb^+$ efflux through veratridine-activated fast sodium channels of differentiated neuroblastoma cells is shown in Fig. 1. One can assume that veratridine activated the rate of rubidium efflux from cells loaded with $^{86}Rb^+$ by 2.5-fold. The addition of NAE with different acyl chains (14:0, 16:0, 18:0) affected the process of veratridine-activated $^{86}Rb^+$ efflux (Fig. 1A,B,C).

All three homologs of NAE caused inhibition of $^{86}Rb^+$ efflux at a concentration of $5 \cdot 10^{-7}$ mol/l. *N*-Palmitoyl- (NAE-16:0) and *N*-stearoylethanolamine (NAE-18:0) in a concentration of $5 \cdot 10^{-6}$ mol/l completely inhibited the veratridine increment of Rb^+

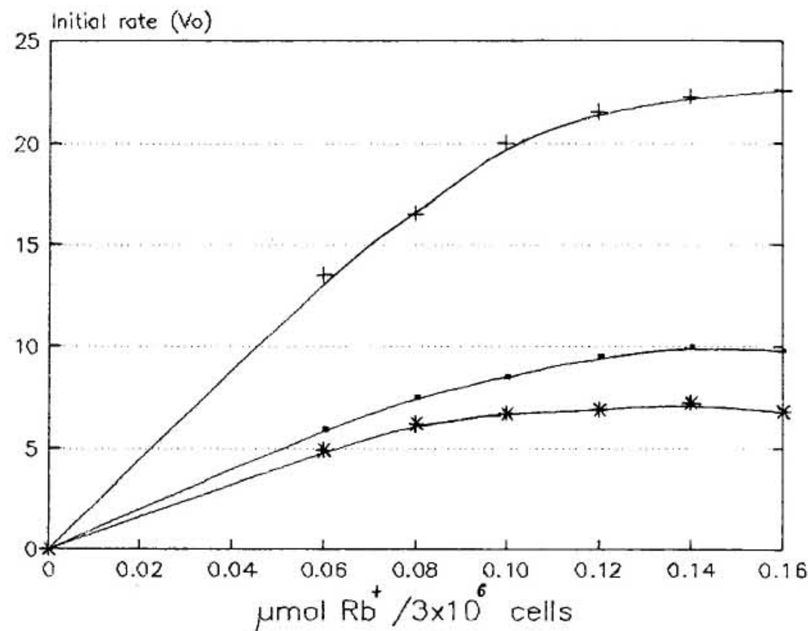

Fig. 2. The influence of NAE-16:0 ($5 \cdot 10^{-5}$ mol/l) on the initial rate of veratridine-activated and basal $^{86}Rb^+$ efflux in neuroblastoma cells. Basal $^{86}Rb^+$ efflux (■); $^{86}Rb^+$ efflux initiated by veratridine (640 μmol/l) (×); effect of NAE ($5 \cdot 10^{-5}$ mol/l) on veratridine-activated $^{86}Rb^+$ efflux (∗). Five different concentrations of $^{86}Rb^+$ were used to preload the cells: $4 \cdot 10^6$, $5 \cdot 10^6$, $6 \cdot 10^6$, $7 \cdot 10^6$ and $8 \cdot 10^6$ dpm of $^{86}Rb^+$ were added into culture medium. The cells accumulated respectively different quantity of label shown at the plot. The initial rates of $^{86}Rb^+$ efflux were obtained as C/t.

efflux from the cells. It is interesting to underline that NAE-16:0, being added to culture medium at concentrations of $5 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ mol/l, decreased Rb^+ efflux to a level lower than basal.

The rate of $^{86}Rb^+$ efflux as a function of NAE concentration is given in Fig. 2. As the character of dependence in initial moment of time is linear, we obtained V_0 as the ratio C/t. So we obtained the second plot which gave us the dependence of the initial rate upon concentration. The value of Rb^+ efflux in the absence of veratridine serves as basal rubidium efflux (Fig. 2).

The results represented in Fig. 2 indicate that NAE-16:0 inhibited both basal rubidium efflux and the transport of Rb^+ through veratridine activated fast sodium channels. The initial rate of rubidium transport in the presence of veratridine and NAE became lower than the basal one.

We also investigated the effect of NAE-16:0 on cation transport through the plasma membrane of neuroblastoma cells in the absence of veratridine. We found that the rate of rubidium influx was higher when NAE-16:0 was added to neuroblastoma cell culture medium (Fig. 3).

Inhibition of the effects of veratridine on cellular lipids by NAE

The addition of veratridine caused the appearance of LPC but did not significantly change the content of all other individual phospholipids or their total content in neuroblastoma cells. NAE in a concentration of $5 \cdot 10^{-6}$ mol/l reduced the effect of veratridine (Table I).

TABLE I

Content of total (nmol / 10^6 cells) and of individual phospholipids (% of total) in untreated neuroblastoma cells or cells treated with veratridine alone or in combination with NAE (n = 7)

Veratridine and NAE were used in concentrations of 10^{-6} mol/l and $5 \cdot 10^{-6}$ mol/l, respectively. Time of incubation in the presence of NAE was 30 min.

Phospholipid	Content of phospholipids in cells		
	Intact	Treated with	
		Veratridine	Veratridine + NAE
Total PL	36.1 ± 0.8	35.8 ± 1.7	36.3 ± 1.2
PC	61.7 ± 2.1	58.4 ± 2.6	62.0 ± 2.4
LPC	–	3.6 ± 0.1	trace
PE	16.3 ± 0.4	17.0 ± 0.5	16.7 ± 0.3
PI	5.1 ± 0.3	5.3 ± 0.3	5.4 ± 0.4
PS	3.8 ± 0.2	4.0 ± 0.2	3.7 ± 0.2
SM	4.1 ± 0.4	3.9 ± 0.2	3.9 ± 0.3
DPG	2.9 ± 0.5	3.0 ± 0.4	2.7 ± 0.2
PG	1.9 ± 0.3	1.6 ± 0.4	1.9 ± 0.3
Other PL	2.5 ± 0.4	2.0 ± 0.2	2.3 ± 0.4
St. zone	1.7 ± 0.2	1.2 ± 0.1	1.4 ± 0.2

In the plasma membrane of neuroblastoma cells veratridine caused considerably more changes of lipid content as compared to the whole cells. The total amount of phospholipids and the amount of PC and PE was decreased as a result of veratridine action; simultaneously LPC and LPE levels were significantly

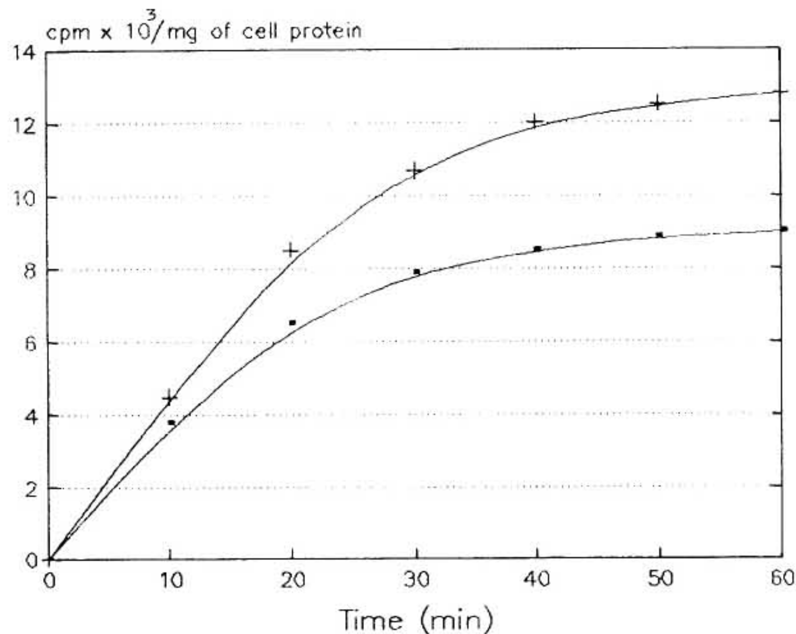

Fig. 3. The effect of NAE-16:0 ($5 \cdot 10^{-5}$ mol/l) on $^{86}Rb^+$ influx in neuroblastoma cells. Basal $^{86}Rb^+$ influx (■); $^{86}Rb^+$ influx in the presence of NAE ($5 \cdot 10^{-5}$ mol/l) (×).

TABLE II

Content of total (nmol / mg of protein) and of individual phospholipids (% of total) in plasma membranes of untreated neuroblastoma cells or cells treated with veratridine alone or in combination with NAE

Veratridine and NAE were used in concentrations of 10^{-6} mol/l and $5\cdot10^{-6}$ mol/l, respectively. Time of incubation in the presence of NAE was 30 min.

Phospholipid	Content of phospholipids in cell plasma membrane		
	Intact	Treated with	
		Veratridine	Veratridine + NAE
Total PL	437 ±14	361 ±11 **	441 ±26 **
PC	44.2± 2.6	33.2± 1.8 *	42.1± 1.8 *
LPC	0.3± 0.1	10.6± 0.4 ***	2.4± 0.2 ***
PE	21.7± 0.7	17.1± 0.3 *	20.9± 0.8 **
LPE	–	5.2± 0.1	trace
SM	7.2± 0.6	6.8± 0.5	7.4± 0.7
PS	8.9± 0.6	9.2± 0.8	8.2± 0.7
PI	9.2± 1.1	9.5± 0.8	9.3± 1.2
Other PL	6.7± 0.4	7.3± 0.7	7.6± 0.5
St. zone	1.8± 0.2	1.1± 0.3	2.1± 0.4

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with intact cells.

TABLE III

Cholesterol (nmol / 10^6 cells), cholesterol ester (pmol / 10^6 cells), free fatty acid (nmol / 10^6 cells) and acylglycerol (nmol / 10^6 cells) content in untreated neuroblastoma cells or cells treated with veratridine alone or in combination with NAE (n = 3)

Veratridine and NAE were used in concentrations of 10^{-6} mol/l and $5\cdot10^{-6}$ mol/l, respectively. Time of incubation in the presence of NAE was 30 min.

Lipid	Content of lipids in cells		
	Intact	Treated with	
		Veratridine	Veratridine + NAE
Cholesterol	18.8±0.6	18.2±0.5	17.8±0.5
Cholesterol ester	25.2±2.2	64.2±0.5 **	29.4±1.9 [b]
Free fatty acids	5.2±0.1	17.7±0.6 ***	6.8±1.9 [c]
Acylglycerol	2.2±0.1	1.4±0.1 *	2.1±0.1 [a]

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with intact cells; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$, compared with veratridine-treated cells.

increased. NAE partly prevented these effects of veratridine on the cell plasma membrane (Table II). The addition of veratridine to culture medium of neuroblastoma cells did not change total cholesterol levels. However, the total amounts of cholesterol esters and of fatty acids was increased. The addition of NAE either normalized their levels or changed them in the normal direction (Table III). It was also found that the total amounts of mono-, di- and triacylglycerols were decreased as a result of veratridine action. NAE prevented these alterations which were caused by the neutrotoxin.

In the plasma membrane of neuroblastoma cells veratridine caused changes similar to the changes in lipid content in the whole cell. The results given in Table IV indicate the time course of lipid changes that occurred in the membrane fraction after addition of

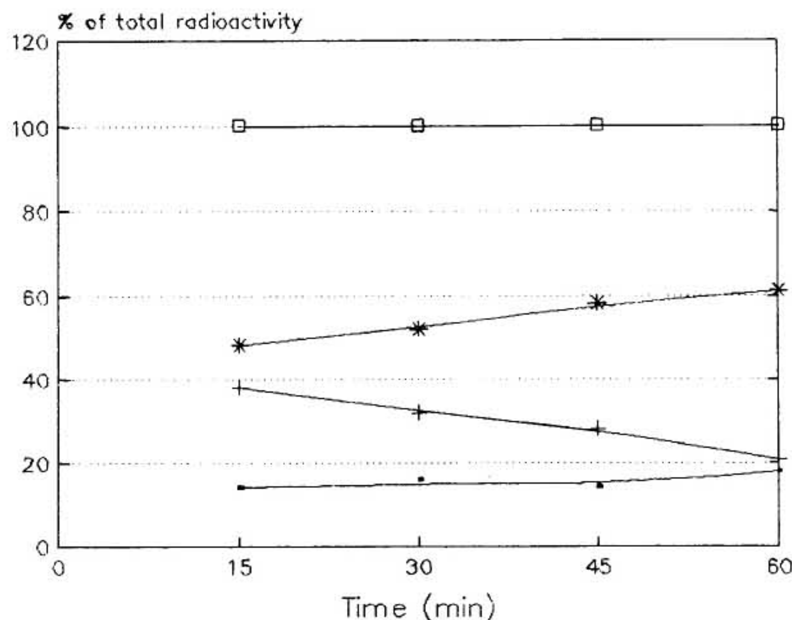

Fig. 4. Time dependence of N-[1-^{14}C]palmitoylethanolamine distribution in subcellular fractions of neuroblastoma cells after incubation in the presence of this compound. The level of label of cytosol (■), plasma membrane (+), microsomal fraction (*) and total radioactivity in the cell (□). Cells were incubated in the presence of $1\cdot10^{-5}$ mol/l of N-[1-^{14}C]palmitoylethanolamine. They were harvested and subcellular fractions were prepared. Total radioactivity of each fraction was counted.

TABLE IV

Time-course of cholesterol (Chol, nmol / mg of protein), cholesterol ester (ECho, nmol / mg), free fatty acid (FFA, pmol / mg), mono- (MG, pmol / mg), di- (DG, pmol / mg) and triacylglycerol (TG, nmol / mg) changes in plasma membrane of untreated neuroblastoma cells and cells treated with veratridine alone or in combination with NAE (n = 5)

The concentration of veratridine and NAE was 10^{-6} mol/l and $5 \cdot 10^{-6}$ mol/l, respectively.

Lipid	Lipid content in plasma membrane of cells						
	Intact	After incubation for					
		5 min		15 min		30 min	
		Veratridine	Veratridine + NAE	Veratridine	Veratridine + NAE	Veratridine	Veratridine + NAE
Chol	271 ± 9	293 ± 14	280 ± 17	268 ± 24	276 ± 13	282 ± 21	287 ± 13
EChol	1.75 ± 0.21	2.39 ± 0.42	1.66 ± 0.34	4.18 ± 0.37 ***	2.07 ± 0.29	7.32 ± 0.34 ***	3.47 ± 0.66 *
FFA	196 ± 24	242 ± 27	182 ± 21	321 ± 46 *	197 ± 34	479 ± 93 *	203 ± 31
MG	94 ± 7	108 ± 7	90 ± 6	143 ± 11 **	97 ± 6	271 ± 36 **	100 ± 11
DG	213 ± 12	241 ± 20	206 ± 18	267 ± 24	218 ± 18	282 ± 47	227 ± 26
TG	31 ± 3	32 ± 2	30 ± 3	22 ± 3	28 ± 3	13 ± 1 ***	23 ± 4

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with intact cells.

veratridine. They show that NAE prevented the development of these changes at all time periods studied.

Distribution of N-[1-¹⁴C]palmitoylethanolamine in the cell

NAE distribution in different subcellular fractions after time periods of 15 to 60 min is represented in Fig. 4. In cytosol the NAE label was increased within 15 min, up to a level which was stable for 1 h. In plasma membrane the highest level of label was found after 15 min of cell incubation with the labeled compound. Later it decreased to a level equal to that of NAE in the cytosol. In the microsomal fraction radioactivity from [1-^{14}C]NAE increased during 60 min of incubation (Fig. 4).

The analysis of distribution of NAE and its metabolites in the plasma membrane is given in Fig. 5. Within the first minutes of incubation NAE accumulated in the plasma membrane. After 15 min of incubation we observed a decrease of its level. By 30 min of incubation we observed an increase of fatty acids. Later their level was decreased and followed by an increase of label in esterified fatty acids.

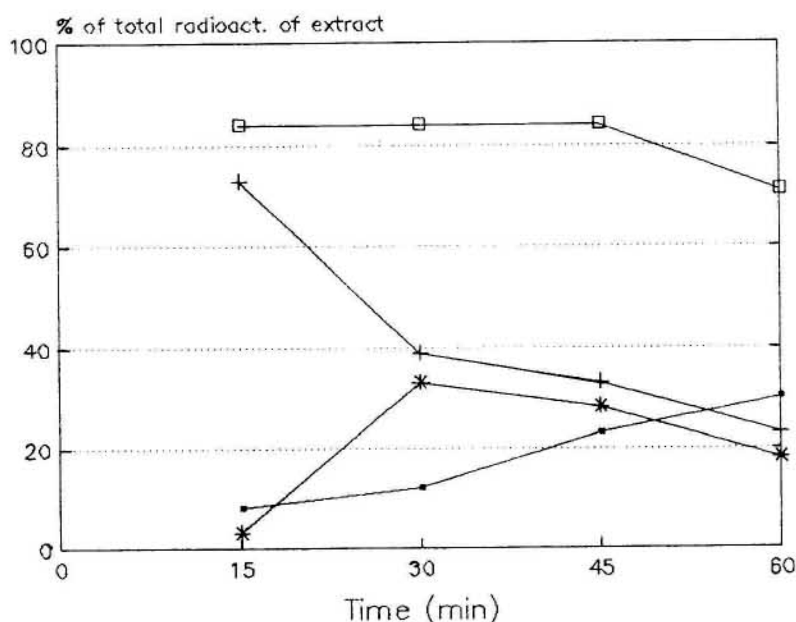

Fig. 5. Time dependence of the contents of N-[1-^{14}C]palmitoylethanolamine and its metabolites in plasma membrane of neuroblastoma cell treated with N-[1-^{14}C]palmitoylethanolamine. The level of label of fatty-acid esters (■), NAE (+), free fatty acids (*) and total radioactivity of metabolites in the cell (□). After incubation in the presence of $1 \cdot 10^{-5}$ mol/l of N-[1-^{14}C]palmitoylethanolamine, cells were harvested and subcellular fractions were analyzed by HRMTLC on silica gel.

It is interesting to note that almost the whole value of radioactivity of NAE which was incorporated into the cell remained in the cell for 1 h (Fig. 4). Part of NAE was degraded. However, approx. 30% of unchanged compound remained in the cell, even after 60 min of incubation (Fig. 5).

Discussion

We assumed that NAE, a minor lipid component of the plasma membrane of undifferentiated neuroblastoma cells, can block cation transport through fast sodium channels. This hypothesis is based on the fact that NAE was found only in undifferentiated cells which have plasma membranes with low excitability [18]. This membrane, in contrast to the excitable one, has no functionally active sodium channels (or only a few) [19].

We have demonstrated that NAE added to cell-culture medium at low concentration is easily incorporated into neuroblastoma cell plasma membrane [31]. In 15 min of cell incubation with [1-^{14}C]NAE the label was found in the plasma membrane, cytosol and microsomal fraction. Part of the label was present in fatty acids and other metabolites of NAE. These results showed that neuroblastoma cells, similar to other neuronal tissues [33], had enzyme systems which catalyse the hydrolysis of NAE. However, in spite of the presence of catabolic enzymes, NAE can stay in the plasma membrane of neuroblastoma cell for relatively long periods of time and can affect membrane function. Even after 1-h incubation approx. 30% of [1-^{14}C]NAE remained unchanged in the plasma membrane.

To investigate the effect of NAE on the function of fast sodium channels we used the biochemical method of Palfrey and Littauer [19] based on the determination of $^{86}Rb^+$ transport through veratridine-activated sodium channels of differentiated neuroblastoma cells. Tetrodotoxin, a neutrotoxin with high specificity for the fast sodium channel, blocked the effect of veratridine on cation transport under our experimental conditions [32]. This fact excluded the possibility of Rb^+ transport through potassium channels and showed that we indeed observed Rb^+ transport through fast sodium channels.

In order to show that NAE prevented veratridine action on cells we used four experimental approaches:

(1) NAE and veratridine were added to the culture medium simultaneously. NAE completely prevented affect of veratridine.

(2) Veratridine was added to the culture medium and 15 min later NAE was added. NAE prevented the effect of veratridine a few minutes after addition.

(3) NAE was added to the culture medium and 15 min later veratridine was added. NAE completely prevented the effect of veratridine.

(4) Cells were incubated for 15 min in the culture medium in the presence of NAE, then the culture medium which contained NAE was discarded and veratridine was added to the cells in a new portion of medium. NAE completely prevented the effect of veratridine.

In the first, the second and the third case NAE and veratridine were present in the culture medium simultaneously.

In the fourth experiment we dealt with NAE pretreatment of cells. As mentioned above NAE added to cell-culture medium is easily incorporated into cell plasma membrane. It remained there for a certain period of time and prevented the effect of veratridine, added in a new portion of medium after the medium which contained NAE was discarded.

Based on these results we selected the first variant of experiments for the data presented here.

NAE-14:0, NAE-16:0 and NAE-18:0, added to the culture medium of neuroblastoma cells in a concentration of $5 \cdot 10^{-7}$ mol/l simultaneously with veratridine, partly prevented activation of fast sodium channels by veratridine. However, the effects of NAE with different acyl chains were not similar.

NAE-16:0 in concentrations of $5 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ mol/l decreased rubidium transport to a level lower than basal. This observation showed that NAE can also affect basal cation transport independently from cation transport through an ion channel. Thus, we investigated the effect of NAE-16:0 on cation transport in the absence of veratridine. NAE did affect this process by activating the Rb^+ influx into cells. We observed different effects of NAE on cation transport in the presence and in the absence of veratridine. Undoubtedly this difference depended on different functional characteristics of intact plasma membranes and plasma membranes loaded with veratridine. Recently, we showed that veratridine modified not only the function of fast sodium channels, but it also changed neuroblastoma cell lipid composition [20]. Veratridine caused the accumulation of LPC and LPE in plasma membrane and the decrease of PC and PE. In the presence of veratridine the total amount of cholesterol esters and of fatty acids was increased. In this paper we have shown that NAE prevented the effect of veratridine on cell lipid content.

It is well known that NAE accumulates in infarcted areas of myocardium [8]. It was shown that long-chain NAE inhibited lipid peroxidation in cardiac mitochondria [33]. These and some other observations [8,9,34] made it possible to suggest that biosynthesis of NAE may constitute an important injury-induced metabolic event aimed at the protection of ischemic myocardial tissue.

These results demonstrate that NAE may be a membranotropic agent which can modify cell func-

tional characteristics and can protect injured cells [16]. Our present data demonstrate that NAE also prevents the effect of the neurotoxin veratridine on neuroblastoma cells.

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