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SURFACE-ACTIVE PROPERTIES OF DIMETHYLETHANOLAMINE AND ITS EFFECT ON ECTO-ATP-ASE ACTIVITY OF PLASMA MEMBRANES

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Dimethylethanolamine (DMEA) belongs to the class of amino alcohols. The compound is widely used in the production of polymers, dyes, and perfumes [3, 6]. As an intermediate in the synthesis of phosphatidylcholine in the liver DMEA plays an essential role in biochemical processes and gives rise to numerous pharmacological effects [8, 9, 11, 15]. The wide spectrum of application of this substance provided the basis for determination of maximal allowable concentrations (MAC) of the amino alcohol in the air of inhabited places in regions where it is manufactured and used.

One of the most sensitive parameters of the cytotoxic effect of chemical compounds is a change in activity of the ecto-enzymes that are located on the outer surface of plasma membranes (PM). Activity of ecto-enzymes is mainly determined by the lipid microenvironment, on which, in turn, toxic substances, especially those possessing surface activity, exert their influence [4, 7, 13].

Guided by the fundamental principles of toxicity, namely that for damage to occur, the toxic substance must interact with the plasma membrane of the cells of the damaged tissue [2], we investigated the surface activity of DMEA, which has not previously been investigated. Using the ecto-ATPase activity of a suspension of smooth-muscle cells as the example, we studied the effect of DMEA on PM function. With this type of experimental approach it is possible to evaluate the reversible and irreversible effects of the mechanism of toxicity.

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EXPERIMENTAL METHOD

To study the surface properties of DMEA we used the method of monomolecular layers [4]. The surface pressure was measured with the aid of a semiimmersed platinum plate, connected to a KhM-6 mechanotron. The boundary potential step was recorded by a dynamic condenser. As electrolyte we used 0.01 M KCl. DMEA was added to the subphase, thus preventing it from directly reaching the phase boundary, and it was tested in concentrations of 10^{-6} to 10^{-2} M, equivalent to $9 \cdot 10^{-4}$-8.9 mg/m^3 in atmospheric air, i.e., the concentration range including values of $MAC_{o.s.}$* (0.25 mg/m^3) and $MAC_{d.s.}$* (0.06 mg/m^3). A solution of azolectin ("Sigma," USA) in chloroform was used to form the phospholipid monolayers.

Smooth-muscle cells were obtained from the rabbit small intestine and guinea pig taenia coli [9, 14]. DMEA-induced changes of membrane permeability were judged by staining the cell suspension with a 1% solution of trypan blue, which does not penetrate into cells through intact PM. The ecto-ATPase activity was determined by the method in [10, 12].

EXPERIMENTAL RESULTS

On the addition of DMEA to the electrolyte, spontaneous concentration of the substance took place on the phase boundary, as shown by an increase in the surface pressure and boundary potential step (Figs. 1 and 2). The system reached the steady state quite quickly (20-30 min). With an increase in the DMEA concentration to 10^{-1} M the surface pressure reached 1.9 mN/m (Table 1), and thereafter showed virtually no change with a further increase in concentration of DMEA. The level of adsorption (Γ) of DMEA on the phase boundary was calculated by Gibbs' equation [1], using the dependence (Fig. 1a):

where R is the universal gas constant, T the temperature, and C the concentration of DMEA (in moles/m^3). This value of adsorption indicates the low surface activity of DMEA and also the fact that the monolayer formed is stretched and the molecules in the monolayer lie a considerable distance apart, as is confirmed by the very low values of the surface pressure.

During adsorption of DMEA from the subphase and the formation of the monolayer structure, an increase of surface potential took place on the surface of the electrolyte, with the "—" sign on the side of the air (Table 1), evidence of a definite orientation of the ionogenic groups of the amine on the phase boundary. The negatively charged oxygen in the DMEA molecule is evidently located nearer the air than the positively charged nitrogen. As the concentration approaches 10^{-1} M and condensation of the monolayer takes place, the negative potential falls, possibly due to reorientation of the DMEA molecules or of their ionogenic groups on the phase boundary.

To understand the processes of interaction of DMEA with membranes we also studied interaction of DMEA with phospholipid azolectin monolayers. On introduction of DMEA into the subphase in concentrations of $1 \cdot 10^{-4}$-$1.6 \cdot 10^{-2}$ M virtually no change took place in the surface pressure and boundary potential step of the azolectin monolayer, and only at the concentration of 10^{-1} M was there a significant change of surface tension — in the course of 20 min the surface pressure of the system rose to 34.4 mN/m (Table 1). Simultaneously with growth of surface pressure of the monolayer, the boundary potential step decreased. This may have been due, first, to reorientation of the dipoles of the lipid molecules on account of interaction of the ionogenic groups of the lipid and the amino alcohol. Second, on introduction of DMEA molecules into the lipid monolayer, the negatively charged oxygen of the molecule may have been located closer to the air (i.e., in the region of positive charges of the lipid molecules), but the positively charged nitrogen was closer to the subphase, which also reduces the boundary potential step. DMEA molecules were inserted into the lipid monolayer evidently in the same way as during monolayer formation from the amino alcohol on the lipid-free surface of the electrolyte.

The results of these investigations thus demonstrate that DMEA possesses surface activity and can form monolayer structures with a boundary potential step of opposite sign (compared with lipid monolayers), in which the orientation of the molecules depends on the concentration of the amino acid. DMEA interacts weakly with the dense azolectin monolayer if concentrations of the amino alcohol are below 10^{-2} M. However, interaction with the monolayer rises sharply with concentrations of the amine of the order of 10^{-1} M, as is shown by changes in surface tension and boundary potential step of the lipid monolayer. These data are important for interpretation of the effect of DMEA on the structure and functions of biological membranes.

*o.s. — oily solution; d.s. — dry substance.

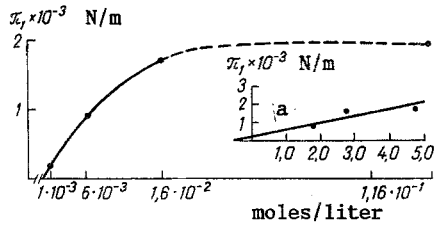

Fig. 1

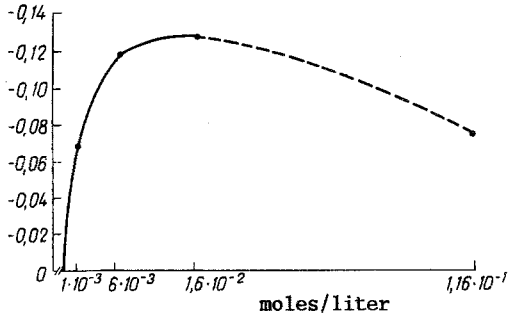

Fig. 2

Fig. 1. Changes in surface tension of monolayer on phase boundary electrolyte — air under the influence of DMEA (a — calculation of level of adsorption of DMEA).

Fig. 2. Changes in potential boundary step on phase boundary electrolyte air under the influence of DMEA.

TABLE 1. Surface Pressure (π) and Potential Boundary Step (U) of DMEA Monolayer and during Interaction of Amino Alcohol with Azolectin Monolayer

DMEA monolayer, DMEA concentration, M	π, mN/m	U, V	Azolectin monolayer,+ DMEA		
			DMEA concentration, M	π, mN/m	U, V
			0,0	17,8	+0,198
0,0	0,0	0,0	$1,000 \cdot 10^{-4}$	18,1	+0,200
$1,00 \cdot 10^{-3}$	0,2	—0,068	$1,000 \cdot 10^{-3}$	18,3	+0,207
$6,00 \cdot 10^{-3}$	0,9	—0,120	$6,100 \cdot 10^{-3}$	18,1	+0,202
$1,60 \cdot 10^{-2}$	1,7	—0,129	$1,610 \cdot 10^{-2}$	18,1	+0,200
$1,16 \cdot 10^{-1}$	1,9	—0,078	$1,161 \cdot 10^{-1}$	34,4	+0,159
			$1,361 \cdot 10^{-1}$	34,0	+0,159

TABLE 2. Effects of Dimethylethanolamine on Permeability for Trypan Blue and Ecto-ATPase Activity of PM of Smooth-Muscle Cells (M ± m)

DMEA concentration in incubation medium, M	Corresponding DMEA concentration in atmospheric air, mg/m^3	Per cent of cells not stained with trypan blue after incubation with DMEA for 15 min	p	Ecto-ATPase activity, per cent	p
0,0	0,0	95±2	—	100	—
10^{-6}	$8,9 \cdot 10^{-5}$	93±1	>0,05	98±2	>0,05
10^{-5}	$8,9 \cdot 10^{-4}$	89±3	<0,05	94±2	<0,05
10^{-4}	$8,9 \cdot 10^{-3}$	91±2	>0,05	66±3	<0,05
10^{-3}	$8,9 \cdot 10^{-2}$	86±4	<0,05	15±3	<0,05
10^{-2}	$8,9 \cdot 10^{-1}$	80±3	<0,05	0,00	—

Since DMEA can interact with phospholipids in the composition of membranes, and since activity of membrane-bound enzymes is determined by the state of the lipid matrix [5, 7, 13], we studied the effect of DMEA on the ecto-ATPase activity of smooth-muscle cells. During incubation of a suspension of smooth-muscle cells for 15 min DMEA in a concentration of 10^{-6} M had virtually no effect on this activity (Table 2).

An increase in the concentration of the amino alcohol in the medium depressed ecto-ATPase activity, but 10^{-2} M DMEA inhibited it completely. After removal of DMEA from the incubation medium, ecto-ATPase activity was only partially restored. At the same time, weakening and blocking of ecto-ATPase activity were not accompanied by significant destruction of PM of the cells, as shown by staining with trypan blue: the number of stained cells was 15% even after incubation with 10^{-2} M DMEA, i.e., with concentrations of the amine completely blocking ecto-ATPase.

Blocking of ecto-ATPase activity of PM by high concentrations of DMEA, because of its surface activity, can be explained as follows. On insertion into the lipid matrix, DMEA condenses the membrane and reduces the boundary potential step on its surface, which, with very high DMEA concentrations, leads to abrupt changes in the lipid microenvironment of the ecto-ATPase, at which it cannot exhibit its activity.

Thus DMEA in concentrations of 10^{-6}-10^{-5} M does not induce any significant changes in activity of ecto-ATPase, sensitive to external influences. The effect of such concentrations is completely reversible. The cytotoxic effect, expressed as a marked decrease and blocking of ecto-ATPase activity, and which is not completely reversible, is manifested at concentrations of the amino alcohol in excess of 10^{-4} M. These concentrations are an order of magnitude lower than those we established for $MAC_{o.s.}$ (0.06 mg/m^3) and almost 30 times less than $MAC_{d.s.}$ (0.25 mg/m^3).

It follows from the data that the cytotoxic effect of DMEA, determined as disturbance of the barrier function of PM and inhibition of ecto-ATPase activity of the smooth-muscle cells, is exhibited at concentrations of the amine below MAC, on determination of which by traditional methods (poisoning the animals for 4 months), changes in the body cannot be recorded. Similar investigations to those described above must therefore be undertaken as a preliminary step for determination of MAC and also for determination of the molecular mechanisms of action of toxic substances on the body. This is all the more important because the undertaking of such investigations is not time consuming, and the apparatus required for the experiments is available in standard Soviet laboratories.

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