MEMBRANE MECHANISMS OF ACTION OF ALKYLATING CYTOSTATICS

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The elucidation of the mechanism of the cytostatic action of alkylating compounds of the chloralkylamine group still remains an urgent problem. Until quite recently, it was predominantly held that they are directly introduced in the genome with the formation of cross-linkages between the nitrogenous bases of complementary DNA sites [10]. However, work has recently been published which casts doubt not only on this hypothesis, but also on the actual fact that alkylating substances penetrate into the cell [11]. Data collected in the literature suggested that the antimitotic activity of antitumor substances can be realized at the level of cascade systems for reception and transformation of an external signal by the cytoplasmic cell membrane.

Accordingly, investigations were undertaken to study interaction of bifunctional alkylating compounds with the principal receptor systems of the cell, coupled with adenylate cyclase (AC) and polyphosphoinositide (PPI) cascades. Three compounds of the chloralkylamine group were studied: dichlorodiethylamine (DCEA), dichlorodiethylmethylamine (embichin), and metaxylyldichlorodiethylamine (MDCEA), to enable the character of the influence of the hydrophobic radical attached to the nitrogen atom on the pattern of interaction of these substances with the test membrane structures to be judged.

EXPERIMENTAL METHOD

Experiments were carried out by radioisotope methods, described fully previously, using a labeled ligand specific for each receptor [5, 6, 8, 9, 13]. The influence of the alkylating compound on the β-adrenoreceptors of the rabbit heart was judged by their displacement of ^{3}H-isoproterenol. Interaction with M_1- and M_2-acetylcholine receptors of the rabbit striatum and heart was assessed by their ability to compete with ^{3}H-quinuclidinylbenzylate (^{3}H-QNB), respectively. To examine the ability of the alkylating agent to bind with α_1-adrenoreceptors of rabbit liver, ^{3}H-prazosin, and with α_2-adrenoreceptors, ^{3}H-clonidine was used. The influence of the alkylating compounds on H_1-histamine receptors of the rabbit cerebral cortex was judged by their ability to compete with ^{3}H-pyrilamine.

AC activity was determined by the method used in [4]. In experiments to study interaction of alkylating compounds with receptors the concentrations of the substances varied from 10^{-8} to 10^{-4} moles/dm^3, as in experiments with adenylate cyclase, from 10^{-6} to 10^{-3} mole/dm^3. Plasma membranes were isolated from the heart, liver, cerebral cortex, and striatum of rabbits by methods described in [4, 7, 9].

The membrane preparation thus obtained were kept at the temperature of liquid nitrogen, and remained active under these circumstances for several months.

Quantum-chemical calculations were done by the SSP MO LKAO Rutan method in the PPDP/2 approximation [12]. Parameters were defined and energy of two-site interactions determined as described in [3].

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TABLE 1. Effect of Alkylating Compounds on Binding of Specific Ligands with Receptors

Type of receptor	Alkylating agents tested		
	embichin	MDCEA	DCEA
	K_i		
α_1	$1{,}55 \cdot 10^{-5}$	$1{,}36 \cdot 10^{-8}$	
M_1	$2{,}10 \cdot 10^{-5}$	$2{,}10 \cdot 10^{-7}$	
H_1	does not interact	$2{,}00 \cdot 10^{-5}$	Does not interact
α_2	$1{,}50 \cdot 10^{-4}$	$0{,}50 \cdot 10^{-6}$	
M_2	—	$1{,}50 \cdot 10^{-7}$	
β	Does not interact		

Legend. K_i) inhibition constant.

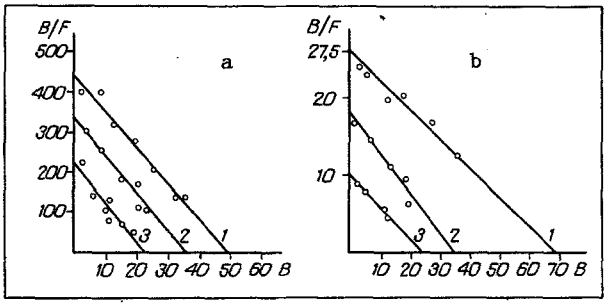

Fig. 1. Scatchard plots to determine quantitative parameters K_{α} and B_{max} for binding of ligands with M_1-cholinergic (a) and α_2-adrenergic (b) receptors. 1) Control (without alkylating compounds), 2) in presence of IC_{50} of MDCEA, 3) in presence of IC_{50} of embichin.

EXPERIMENTAL RESULTS

The experiments showed that none of the alkylating compounds, in the concentrations tested, interacted with β-adrenoreceptors or affected adenylate cyclase activity connected with them. DCEA interacted with none of the other receptors tested. MDCEA had marked ability to interact with the group of receptors activating the PPI cascade: α_1-adrenergic, M_1-cholinergic, H_1-histamine. The same compounds also had affinity for receptors inhibiting adenylate cyclase activity: α_2-adrenergic and M_2-cholinergic (Table 1). Embichin had basically the same spectrum of points of application but much weaker activity. Unlike MDCEA, embichin did not interact with H_1-histamine receptors, and inhibited binding of ^{3}H-QNB with M_2-cholinergic receptors by only 14%, and only in a concentration of $3 \cdot 10^{-4}$ moles/dm^3, thus ruling out any question of specificity of the substance for receptors of this type (Table 1).

To study the character of interaction of the alkylating compounds with receptors experiments were carried out to determine the effect of these substances on the dissociation constant (K_{α}) and the maximal number of binding sites (B_{max}) of specific ligands on particular types of receptors. It was found that during interaction between alkylating compounds and receptors K_{α} of the markers remained virtually unchanged whereas B_{max} fell by 50-65%. As an example we show Scatchard plots for two types of receptors: M_1-cholinergic, activating the PPI-cascade, and α_2-adrenergic, inhibiting adenylate cyclase (Fig. 1). This fact can be regarded as evidence of direct competition between the alkylating compounds and the markers used for binding sites on the receptors.

The results are evidence in support of the hypothesis mentioned above that the antimitotic activity of alkylating compounds is utilized as early as at the membrane level, within the limits of two universal regulating systems: adenylate cyclase (AC) and PPI, responsible for the reception, transformation, and realization of external signals by the cell [14]. Signals inducing proliferation are conducted and realized by the PPI system. Activation of the AC system, on the other hand, inhibits cell division

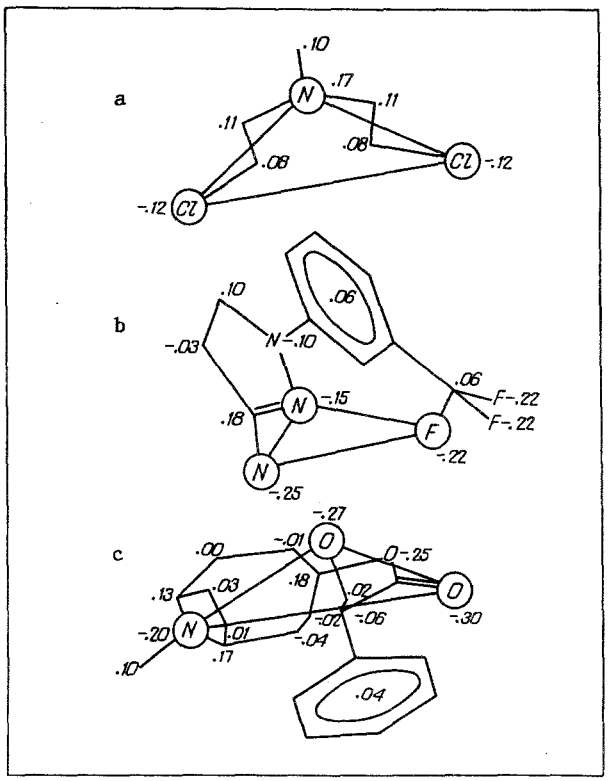

Fig. 2. Models of physiologically active conformations of inhibitors of the PPI system. a) embichin, b) BW755C, c) atropine. Triangle isolates universal pharmacophore grouping consisting of three PAC.

[14]. The facts discovered in the present investigation, namely blocking of receptors activating PPI system (M_1-cholinergic, α_1-adrenergic, H_1-histamine) and binding with receptors inhibiting AC activity (α_2-adrenergic, M_2-cholinergic), are undoubtedly important for realization of the cytostatic effect of alkylating agents.

On the other hand, the similarity of the points of application of the chloralkylamines with M-cholinolytic and α-adrenolytic agents, blockers of H_1-histamine receptors and also, as the writers described previously [2], inhibitors of cyclo- and lipoxygenases, suggests that the physiologically active substances mentioned above possess certain common structural features.

Quantum-chemical calculations showed that this common structure may consist of the basically similar arrangement of the proton-acceptor centers (PAC). Virtually all xenobiotics of the above-mentioned groups have three PAC with charges of between -0.15 and -0.30, lying on one side of the hydrophobic part of the molecule and forming a triangle with sides measuring 2.5-5.3, 2.5-5.3, and 5.3-7.6 A (Fig. 2). This enables the molecule of the physiologically active substance to be fixed by weak nonvalency interactions to the regulated region of the protein macromolecule, affecting the conformation of the active center. Considering general trends of action of these compounds within the limits of the regulating cascade, the active structure of the molecule thus revealed can be interpreted as a universal "pharmacophore," blocking PPI-dependent processes.

The elements of specificity in the general pattern of action of each concrete preparation are evidently determined by the structure of the hydrophobic part of the molecule, the size and conformational lability of which are responsible for the degree of penetration into the cell, and thus may determine their pharmacokinetic characteristics.

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IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF THE SPECIFIC α_2-GLOBULIN OF HUMAN GRANULOCYTES

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Polymorphonuclear leukocytes, or granulocytes, are universal effector cells whose potential is largely determined by their ability to release biologically active substances stored in the granules of these cells [10, 12, 13]. Granulocytes play a key role in defense against infections, in tissue damage and the development of vascular changes during inflammation, and in antitumor defense. Specific proteins secreted by granulocytes activate proteolytic cascade systems in the blood plasma [2, 11], interact with receptors on the surface of immunocompetent cells [2, 10], participate in the regulation of hematopoiesis [10] and in disturbances of blood clotting and fibrinolysis [11], and they possess powerful collagenolytic potential [13].

The list of granular proteins of neutrophils includes antimicrobial cationic components — myeloperoxidase, lysozyme, and lactoferrin, and neutral proteinases — elastase, cathepsin G, collagenase, gelatinase, B_{12} binding protein, transcobalamin, etc. More recently several new granulocytic proteins have been described [3, 4, 14, 15].

The aim of this investigation was to identify the specific α_2-globulin of human granulocytes and to undertake their immunochemical and physicochemical study.

EXPERIMENTAL METHOD

To obtain antisera rabbits were immunized with an extract of leukocytes and the antisera were adsorbed with blood plasma from healthy donors and with extract of liver tissue, under the control of immunodiffusion analysis with polyspecific antisera against human plasma proteins and liver extract. Leukocytes were isolated from whole blood, and pus was obtained by opening an abscess. The method of preparation of the leukocyte lysate and extracts of pus and tissues was described previously [4, 9].

Antisera against leukocytic elastase, lactoferrin, lysozyme, fibronectin, amyloid P-component, C-reactive protein (from "Calbiochem," USA), and standard test systems for soluble leukocytic antigens [4], obtained at the Research Institute of Physico-

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