Effect of Ionenes on Ion Permeability of Erythrocyte Membranes

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Ionophore activity of ionenes, like that of polymyxins, is shown to be potentiated by long-chain antiions. Rapid stoichiometric anion equilibration in isoosmotic buffered sugar medium revealed that the amplitude of Cl^-/OH^- exchange in erythrocytes induced by an ionene-detergent complex gradually drops with the decrease of both the charge density and the number of monomeric moieties in the polymeric chain. This is also paralleled by passive K^+ leakage from the cells.

Key Words: *polycations; erythrocytes; membranes; passive ion permeability*

Numerous polycations, both natural and synthetic, possess high biological activity [2,10,12,14]. However, the mechanisms underlying this property remain poorly understood. In this context, studies on the correlation between the structure and activity of polymers are of great interest and will probably help optimize the search for new medical preparations. Ionenes are a group of polycations with high biological activity [4,10-12]. They represent linear polymers positively charged due to quaternary nitrogen atoms in the macrochain backbone [8]. At the same time, polymyxins, a group of multipositively charged antibiotics, act by affecting the ion permeability of membranes [5,14]. An essential feature of their action on the membrane is that ionophore activity is potentiated by long-chain antiions (fatty acid and detergent) [6,7]. It may be speculated that natural and synthetic polycations affect negatively charged biological membranes through similar mechanisms. However, synthetic polycations are more convenient for model experiments due to the variability of the chain length and the charge-to-charge distance.

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The objective of the present study was to investigate the ability of ionenes to modify the passive ion permeability of biological membranes as a possible mechanism of their cytotoxicity and to elucidate the dependence of this process on the charge density and molecular weight of the test substances.

MATERIALS AND METHODS

Ionenes were kindly supplied by the Department of High-Molecular Compounds, M. V. Lomonosov Moscow State University. Erythrocytes were isolated from citrate rat blood (1:9, 3.8% citrate:blood) by centrifugation at 1200 g and 4°C for 5 min. The erythrocytes were washed twice with 5 volumes of NaCl (149 mmol/liter) in Tris buffer (5 mmol/liter, pH 7.4). The washed erythrocyte suspension was stored at 8°C. Efflux of Cl^- ions was assessed by measuring the rapid acidification amplitude using a glass electrode for pH measurements (Radelkis, Hungary) as described earlier [14]. Potassium was measured with a selective membrane electrode (Radelkis). The output signal was received by pH-meters working as millipotentiometers and recorded. The electrode was calibrated by the method of

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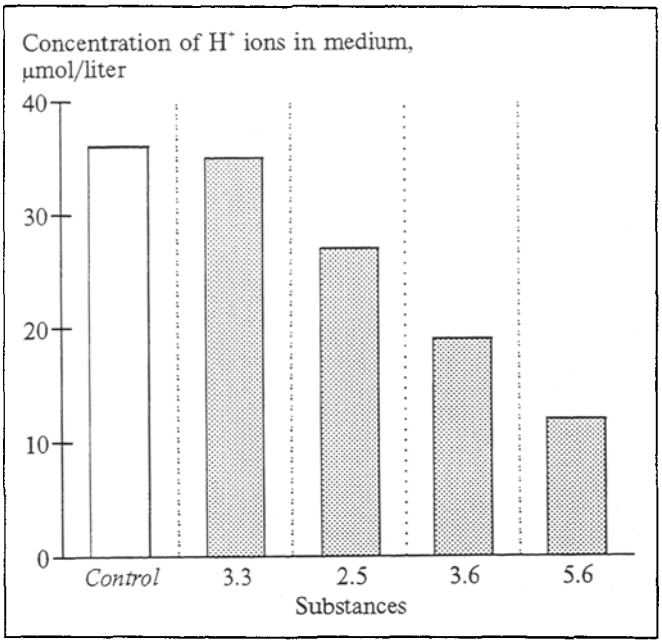

Fig. 1. Amplitude of Cl^-/OH^- exchange across erythrocyte membrane as a function of charge density on polycation in medium containing 2×10^{-5} mol/liter SDS. Concentration of 2.5–ionene (av.mol.weight 7000) 0.014×10^{-5} mol/liter; concentration of 3.3–ionene (av.mol.weight 12,000) 0.016×10^{-5} mol/liter; concentration of 3.6–ionene (av.mol.weight 12,000) $0.019\times\times10^{-5}$ mol/liter; concentration of 5.6–ionene (av.mol.weight 12,000) 0.02×10^{-5} mol/liter. Concentration of all ionenes by quaternary nitrogen 10^{-5} mol/liter.

standard adds [3]. The concentration of erythrocytes in the cuvette was about 20,000/μl. The incubation medium contained 300 mmol/liter sucrose, 0.4 mmol/liter KCl, and 5 mmol/liter Tris, pH 7.4. The test compounds were added to the incubation medium before the addition of erythrocytes. The results were processed statistically using the Wilcoxon-Mann-Whitney test.

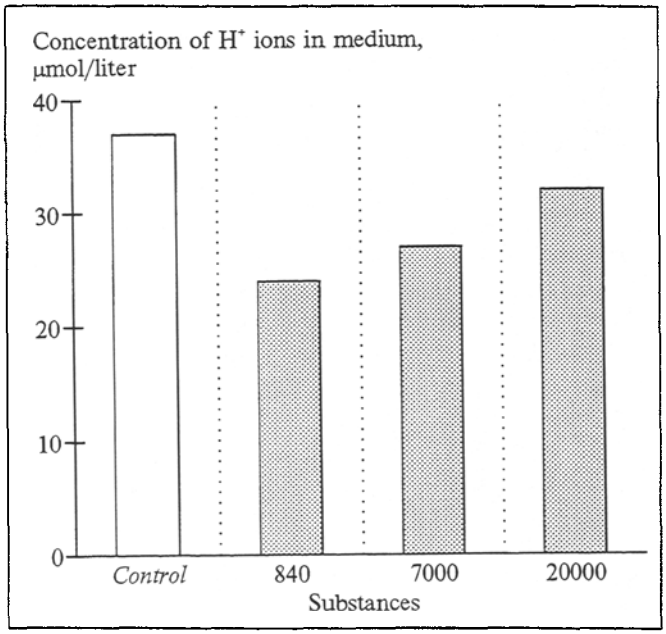

Fig. 2. Amplitude of Cl^-/OH^- exchange across erythrocyte membrane as a function of chain length on polycation in medium containing 2×10^{-5} mol/liter SDS. Number of monomeric moieties of 2.5–ionene with a molecular weight of 840=4; 7000, about 26; 20,000, about 72. Concentration of all ionenes by quaternary nitrogen 10^{-5} mol/liter.

RESULTS

A study of rapid stoichiometric equilibration (Cl^-/OH^-) in isoosmotic buffered sucrose medium revealed no effect of ionenes (up to 1 μmol/liter, i.e., in nonagglutinating concentrations) on transmembrane ion transfer, whereas in combination with SDS all test polycations reliably reduced the amplitude of Cl^-/OH^- exchange in the erythrocytes (Figs. 1 and 2).

At equal concentrations in monomeric moieties the effect of an ionene-detergent complex on the amplitude of Cl^-/OH^- exchange in erythrocytes depends on the charge density in the backbone of the test polycation molecules (Fig. 1). Ionenes with a lower charge density, i.e., with a greater number of $-CH_2-$ groups between quaternary nitrogens, had a more pronounced effect on the amplitude of Cl^-/OH^- exchange in erythrocytes. The maximal effect was exhibited by the complex of SDS with 5.6-ionene with a minimal charge density (5.5 $-CH_2-$ groups per positive charge), whereas 3.3-ionene (3 $-CH_2-$ groups per positively charged nitrogen) was less effective: the amplitude of Cl^-/OH^- exchange was close to the control value.

The effect of an ionene-detergent complex on the amplitude of Cl^-/OH^- exchange in erythrocytes in an equal monomeric moiety concentration depends not only on the charge density, but also on the length of the polymer. For example, for 2.5-ionene the amplitude of anion exchange reliably declines with the decrease in the number of monomeric moieties (Fig. 2), i.e., short chains more effectively modify Cl^-/OH^- exchange in erythrocyte membranes.

The observed dependences on the chain length and charge density of the polymer correlate with previous reports on the acute toxicity of these substances [4]. This suggests that the disturbances in membrane permeability may partially underlie the toxic effect of polycations.

The Cl^-/OH^- exchange was paralleled by a passive leakage of potassium ions from cells, which was not observed under the action of SDS and ionenes alone in the same concentrations. The efflux of K^+ ions was 134±19 μmol/5 min. Passive K^+ leakage is known to be accompanied by Cl^- import [1]. The decreased amplitude of anion exchange, which is mediated through a special system of ion-transport

proteins [13], may therefore result from ion bypass (or additional consumption of Cl^- for coupling) due to the appearance of an additional channel of ion leakage. The complexes formed from detergent and polyion are apparently more "aggressive" than the original polycation, which manifests itself in a disturbed ion permeability of the membrane. In the uncharged hydrophobic (due to hydrocarbon SDS chains) form ionene more easily becomes incorporated into the cell membrane, thus affecting its structural integrity, in a way similar to that for a polymyxin-detergent complex [5].

Thus, ionenes used in combination with negatively charged detergent disturb the ion permeability of erythrocyte membranes, while neither ionenes nor detergent alone within the studied concentration range affected membrane ion permeability. It may be concluded that upon interacting with such antiions, polycations acquire the properties of a membrane-damaging agent. The effect of such an agent on the membrane is presumably related to the geometry (or the lipo-hydrophilic balance) of the polyion-antiion complex, since it depends on the size of molecules, the charge density on the polycation, and probably, as for polymyxins, on the chain length of the antiion. It may be hypothesized that ionenes, like polymyxins [9], when interacting with antiions form ion channels in the membrane which are composed of several molecules of polymer and detergent.

It may be assumed that the disturbance of membrane ion permeability induced by ionenes is a manifestation of their cytotoxicity. If so, the cytotoxicity of an ionene should depend not only on the affinity of the polyion to the target cells but also on the presence of long-chain antiions (for example, free fatty acids) in the membrane.

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