The Efficacy of Bronchodilators Is Determined by Electrostatic Interactions

I. A. Sitarchuk, B. Kh. Yagmurov, S. K. Soodaeva, and A. G. Chuchalin

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Donnan potential recordings demonstrate that the preparation troventol exhibits a positive surface charge, atropine sulfate a negative surface charge, and atrovent a charge close to zero. Analysis of electrostatic interactions between these preparations and cells reveals that alveolar macrophages exhibit a more negative surface charge than peritoneal macrophages. More pronounced electrostatic interactions of the preparations with membrane macrophages are observed with troventol versus the other broncholytics studied.

Key Words: *bronchodilators; troventol; electrostatic interactions; macrophages*

The search for and selection of new preparations for bronchospasm continue to be pressing problems, even though certain advances have been made in the treatment of bronchial asthma and obstructive pulmonary diseases. Since activation of cholinergic mechanisms is one of the main stages in the pathogenesis of bronchospastic syndrome, it has become traditional to use anticholinergic preparations. A study of the physical and chemical properties of these preparations is of great interest for unraveling the mechanisms of interaction of widely used anticholinergic preparations with cells and tissues.

An attempt has been made here to assess the possible electrostatic interactions between some cholinergic preparations (atropine sulfate, atrovent, and troventol; Fig. 1) and target cells (macrophages).

MATERIALS AND METHODS

For alveolar lavage, 10 ml of Hanks solution were introduced via a cannula into the trachea of a narcotized rat (hexobarbital, 34 mg/ml, 1 ml intramuscularly). The fluid was thoroughly washed twice, taken into a syringe, and filtered through double nylon mesh.

Alveolar macrophages were isolated by two-time centrifugation (400 g, 10 min) of bronchial lavage. The pellet was washed off twice and resuspended in 10 mM NaCl in 1 mM Tris-HCl buffer (Sigma), pH 7.4, and kept at the temperature of thawing ice. Cells were counted in a Goryaev chamber.

Isolation of peritoneal macrophages was performed after Berton [3]. The charge of the preparations and cells was determined by recordings of the Donnan potential generated by the agents or cell suspensions using the technique of Ojteg [5] with some modifications [1,2,4]. Figure 2 represents a scheme of the experimental set-up for measuring the Donnan potential. The radius of the cuvette, separated from the solution by a semipermeable membrane, was 10 mm; the semipermeable membrane was permeable for molecules of molecular weight less than 12 kD. Ten mM NaCl in 1 mM tris-HCl buffer (pH 7.4) served as the solvent for both compartments (cuvette with the agent to be determined and the surrounding bath). Prior to the start of the experiment the cuvettes with semipermeable membranes were soaked in 3 liters

Research Institute of Pulmonology, Ministry of Health of Russian Federation, Moscow

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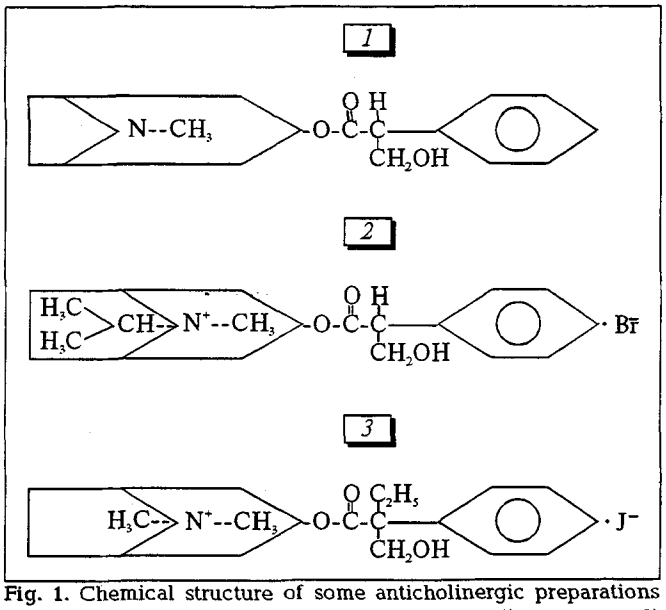

Fig. 1. Chemical structure of some anticholinergic preparations used in pulmonology. *1*) atropine sulfate; *2*) atrovent; *3*) troventol.

of the solution for 48 hours. The volume of preparation solution or cell suspension (placed in the cuvette) was 1 ml, and the volume of outside solution was 1 liter. The temperature of the solution was within the range of 20-25°C. The potential was measured with two potentiometric chlorosilver electrodes (OP-0820P, "Radelkis") and recorded by means of a TR 1676, EMG 1469 programmed voltmeter (Hungary), which was programmed by an EMG 666/B calculator (Hungary). The resistance between the electrodes was about 10 kΩ, and the voltmeter input resistance was 1 MΩ. The preparation concentration was 2-5 mg/ml, and the number of cells was 1-6 mln/ml.

The Donnan potential was measured in the following manner: first the reference potential was

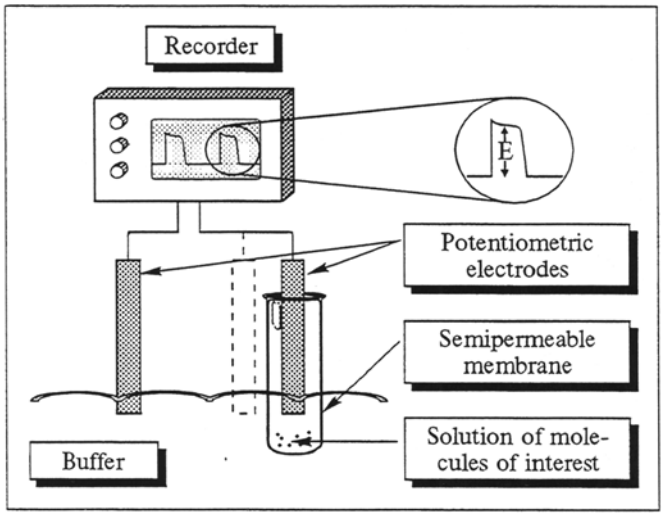

Fig. 2. Experimental set-up for determination of preparations and cells by Donnan potential measurements.

measured, i.e., two electrodes were immersed in the outside solution and during 5 min the EMG 666/B recorded 500 measurements of the potential difference between the electrodes. The mean value and variance were then estimated and the result was printed out. After this one of the electrodes was immersed in the solution of the agent studied and the potential was measured for 5 min, this being followed by determination of the reference potential. The cycle was repeated at least twice. The difference between the mean values of the agent potential and the reference potential was used to calculate the charge. Nonpermeating polyelectrolytes located on one side of the semipermeable membrane influence the distribution of mobile ions. At equilibrium the diffusible ions will be unequally distributed (Donnan effect). The Donnan potential (E) for a single monovalent ion is equal to:

$$E = (RT/ZF) \times \ln(C_i/C_o), \quad (1)$$

where R is the universal gas constant (8.314 J/K×mol), T is the absolute temperature, Z is the charge and C the concentration of mobile cations and anions, and F is the Faraday constant (96487 C/mol). The indexes "i" and "o" designate inside and outside solutions with respect to the cuvette.

The electroneutrality of the inside solution (taking into account the charge of the agents determined) gives us:

$$Z_p C_p = C_i \text{ (cations)} - C_i \text{ (anions)}, \quad (2)$$

where Z_p and C_p are the charge and concentration of the preparation, respectively.

The large volume of the outside solution compensates for the small number of ions transported inside the cuvette. It can be written with great precision that:

$$C_o \text{ (cation)} = C_o \text{ (anion)}, \quad (3)$$

Combining equations (1), (2), and (3) yields an equation for the charge of the preparation of interest:

$$Z_p = (C_o(\text{cation})/C_p)\ (\exp(EF/RT) - \exp(-EF/RT), \quad (4)$$

RESULTS

Study of the net surface charge of troventol preparation showed that the preparation exhibits a positive surface charge equal to 0.01±0.005 (Table 1). We determined the net charge of other anticholinergic preparations for comparative analysis: atropine sulfate was found to show a negative charge (-0.14±0.01) and atrovent showed a zero charge within the precision range of the measurements.

TABLE 1. The Donnan Potentials and Charges of Preparations and Cells ($M \pm m$)

Agent assayed	The Donnan potential, mV	Z, elementary units
Atropine sulfate	-1.80 ± 0.10	-0.14 ± 0.01
Atrovent	0.03 ± 0.09	0.003 ± 0.01
Troventol	0.12 ± 0.06	0.01 ± 0.005
Peritoneal macrophages	-2.01 ± 0.1	$(-3.14 \pm 2.2) \times 10^{11}$
Alveolar macrophages	-2.45 ± 0.11	$(-9.3 \pm 3.8) \times 10^{11}$

To study possible electrostatic interactions of the preparations with the cells we measured the net surface charge of peritoneal and alveolar macrophages. The cell charges are given in the table. Alveolar macrophages demonstrated a more negative charge than peritoneal macrophages.

Electrostatic interactions of negatively charged macrophages with the preparations may lead to different consequences. For instance, in the case of troventol there will be an increase in the concentration of the preparation in the vicinity of the macrophage cell membranes due to the Coulomb attraction of positively charged troventol molecules by negatively charged macrophages.

The opposite process will be observed in the case of atropine sulfate, due to the Coulomb repulsion of the same charges. Electrostatic interactions were found to be insignificant for atrovent.

Thus, a more marked electrostatic attraction of the preparation molecules to macrophage membranes is observed with troventol versus other broncholytics.

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