Mechanism Underlying the Effect of the Bronchodilator Troventol on Histamine Secretion

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Molecular mechanism of the effect of the anticholinergic bronchodilator troventol on histamine secretion, the initial step in bronchospasm, is studied. Atrovent (ipratropium bromide) and atropine sulfate are used as reference preparations. Histamine secretion is induced by adding phorbol myristate acetate to cell suspension. In cells incubated for 5 min with troventol histamine secretion constitutes 52.4% of the maximum level, while atrovent and atropine have no effect on this process. Histamine secretion in mast cells is initiated by a sharp increase in cytosolic calcium. Troventol and atrovent reduce the initial rate of passive calcium entry into the cells by 56.3 and 28%, respectively, while atropine does not affect this parameter.

Key Words: *cholinolytics; troventol; histamine; liposomes; microviscosity*

The new Russian-manufactured bronchodilator troventol, iodmethylate of α-hydroxymethyl-α-phenylbutyrate tropine ester, effectively prevents experimental bronchospasm induced by cholinomimetics, due to its high selectivity for tracheal and bronchial M-cholinoreceptors. This is probably the reason why troventol is ineffective in histamine-induced in contrast to carbachol-induced bronchospasm. Histamine is a mediator of inflammation and its release in lungs can induce bronchospasm [2]. Our previous data suggest that troventol exhibits lung-selective anti-inflammatory activity [3], therefore we assumed that its antihistamine effect is mediated through inhibition of histamine secretion rather than through blockage of histamine receptors in tracheal and bronchial smooth muscles.

The aim of the present study was to elucidate the mechanism of the effect of troventol on histamine secretion in the peritoneal exudate cells. Mast cells is an obligatory component of peritoneal exudate. Atrovent and atropine sulfate, bronchodilators of the cholinolytic family, served as the reference drugs.

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MATERIALS AND METHODS

Random-bred albino rats weighing 180-200 g were used. Cells were routinely isolated from peritoneal exudate as described elsewhere [4], and cell suspension containing more than 90% live cells (judging from the trypan blue exclusion test) was used.

Cell suspension (2×10^6 cells/ml Hanks' medium) stored on ice was incubated for 5 min at 37°C for functional recovery. Troventol, atrovent (ipratropium bromide), and atropine sulfate were added to the incubation mixture. In all samples, except control, histamine secretion was induced by incubating the cells with 10^{-6} mg/ml phorbol myristate acetate (PMA, Sigma) [10] for 5 min with constant shacking. The effect of PMA alone was taken as the maximum level of histamine secretion. Incubation was stopped by adding 1 ml ice-cold Hanks' medium containing 2 mM EDTA (Sigma) and centrifugation for 10 min at 1500*g*. The concentration of histamine (Sigma) in the supernatant was determined spectrophotometrically by the reaction with o-phthaldialdehyde [11].

Passive cell membrane permeability for Ca ions was assessed by the inclusion of the fluorescent Ca indicator Quin 2-AM (Sigma). The cells were first

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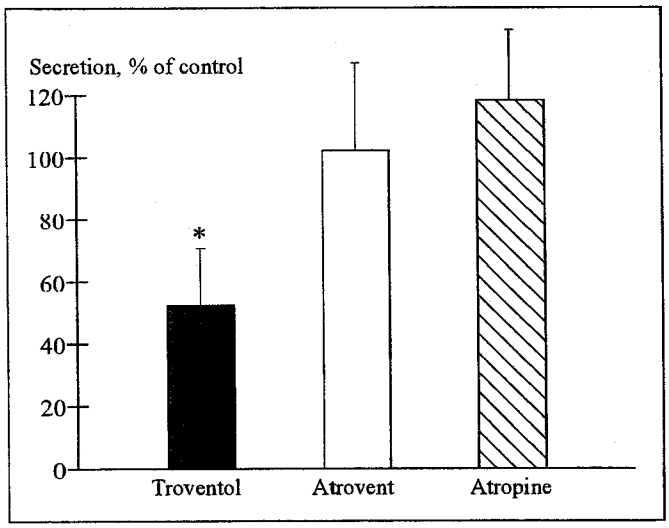

Fig. 1. Effects of troventol, atrovent, and atropine on histamine secretion in peritoneal exudate cells. The agents are added in a concentration of 10^{-3} mg/ml. Maximum level of histamine secretion induced by phorbol myristate acetate minus spontaneous secretion is taken as 100%. *$p<0.05$ compared with the control.

washed with Ca^{2+}-free Hanks' medium and incubated with Quin 2-AM (2 μmol/10^6 cells) for 60 min at 37°C. Twice washed cells were then suspended in Ca-free Hanks' medium. Calcium entry was induced by adding 1 mM $CaCl_2$ to the incubation medium. The initial rate of Ca^{2+} entry into the cells was determined from the slope of the fluorescence intensity curve [9].

Triton X-100 (Serva) in a final concentration of 0.05% was added to samples after each measurement for cell lysis and determination of the maximum binding of Ca^{2+} with Quin 2-AM. Minimum binding was measured after adding 0.5 mM $MnCl_2$. Intracellular calcium concentration was routinely calculated as described elsewhere [5].

Microviscosity of cell membranes was evaluated using the hydrophobic fluorescent probe pyrene (2.5 μmol/10^6 cells, Fluka) by the pyrene monomer-to-excimer fluorescence ratio (F_m/F_e) [1]. Fluorescence spectra were recorded on an MPF-44A spectrofluorimeter (Perkin Elmer) at 37°C. Nonspecific effect of the test preparation on the cell membrane microviscosity was studied on liposomes prepared from egg yolk lecithin—cholesterol equimolar mixture (both from Sigma) [1]. The samples contained 0.05 mg/ml lecithin per 5 μM pyrene.

Other reagents of chemically pure or extra pure grades were manufactured in Russia; Hanks' solution was prepared from Russian-manufactured reagents of the above-indicated purity except of glucose (Merck).

The data were processed statistically using nonparametric Wilcoxon—Mann—Whitney test.

RESULTS

Histamine secretion in PMA-stimulated peritoneal cells surpassed the spontaneous secretion by 20%. Unlike atrovent and atropine, troventol in a concentration of 10^{-3} mg/ml inhibited histamine secretion by 47.6% of the maximum level.

Histamine secretion to strongly depends on intracellular Ca^{2+} concentration, which in its turn depends on the plasma membrane permeability for calcium ions [12]. In light of this it seems interesting to find out whether troventol modulates this important stage of the secretory process. We have found that troventol (10^{-3} mg/ml) and atrovent reduce the rate of passive calcium entry by 56.3 and 28%, respectively, while atropine has no effect on this parameter (Fig. 2 and 3). On the other hand, all test drugs did not change Ca capacity of the cytosol, as the difference between minimal and maximum cell Ca^{2+} concentration remained unchanged (131±10.0 nM in the control, 128.8±7.0 nM for troventol, 129.0±11.0 nM for atrovent, and 126.0±11.5 nM for atropine). It seems

TABLE 1. Effect of Bronchodilator on Membrane Microviscosity in Peritoneal Exudate Cells and Liposomes (*M±m*)

Preparation, mg/ml		Cell membranes		Liposome membranes	
		F_m/F_e	F_m/F_e+PMA	F_m/F_e	F_m/F_e+PMA
Control		3.44±0.12	3.76±0.10*	4.26±0.16	3.89±0.11*
Troventol	10^{-3}	3.77±0.15*	4.03±0.12*	4.00±0.11*	3.58±0.13*
	10^{-6}	3.82±0.16*	4.05±0.13*	—	—
Atrovent	10^{-3}	3.70±0.13*	3.88±0.11*	4.04±0.15*	3.72±0.16*
	10^{-6}	3.58±0.17	3.72±0.13	—	—
Atropine	10^{-3}	3.79±0.12*	4.05±0.14*	3.83±0.13*	3.56±0.12*
	10^{-6}	3.78±0.11*	3.98±0.13*	—	—

Note. F_m/F_e — monomer-to-excimer fluorescence ratio. *$p<0.05$ compared with the control. In liposomes only one concentration of the preparations was studied.

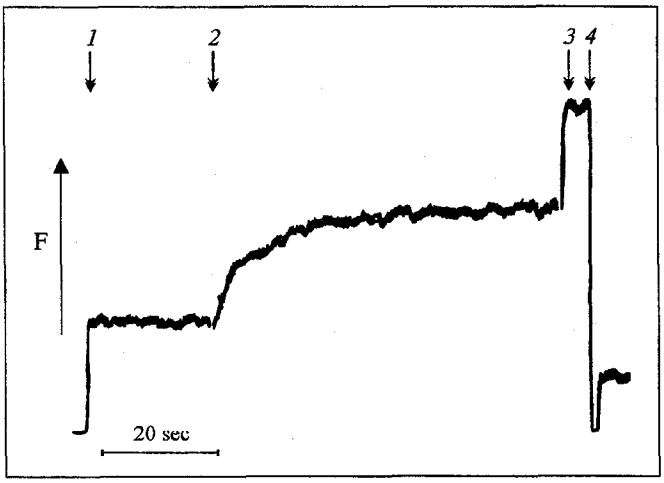

Fig. 2. Typical record of Ca^{2+} entry into cells. F: intensity of Quin 2-AM fluorescence in relative units. Arrows indicate addition of 2×10^6 cells (*1*), 1 mM $CaCl_2$ (2), 0.05% Triton X-100 (3), and 0.5 mM $MnCl_2$ (*4*).

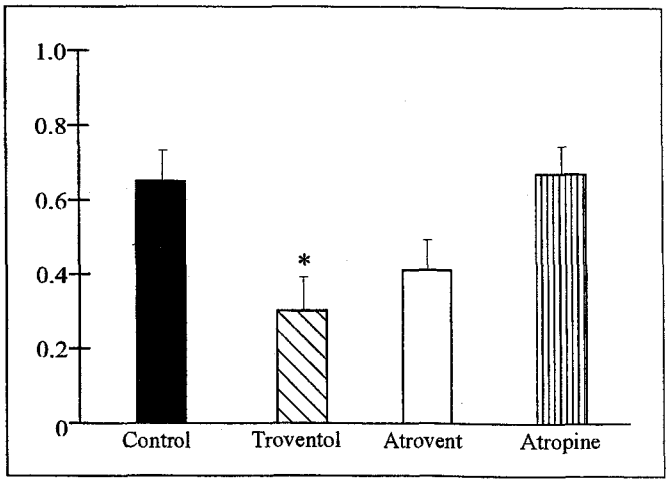

Fig. 3. Effects of troventol, atrovent, and atropine, on passive Ca entry into the peritoneal exudate cells.
Ordinate: initial rate of Ca^{2+} entry into cells, nmol/min×10^6 cells.

likely that the test drugs modulate physicochemical properties of plasma membrane.

Activity of Ca channels and Ca exchangers responsible for membrane permeability for Ca ions directly depends on their lipid surrounding [6]. The study of membrane microviscosity with pyrene incorporated into lipid bilayer showed that all test drugs to the same extent increased the F_m/F_e ratio directly proportional to microviscosity [8], the effect of troventol and atrovent being dose-independent in the studied concentration range (Table 1). The effects of the test drugs on physicochemical characteristics of lipid bilayer were studied using liposomes as the model of hydrocarbon phase of cell membranes. Despite the differences in chemical structure all test preparations slightly reduced the microviscosity of liposome membranes (Table 1). It can be hypothesized that these agents nonspecifically interact with lipid bilayer in liposomes, while their effect on whole cells is more specific.

Troventol had a more pronounced effect on Ca^{2+} entry and histamine secretion than atrovent and atropine. This is probably due to higher selectivity of troventol to M-cholinoreceptors. Since cholinoreceptors are functionally coupled with chemosensitive Ca channels [7], troventol can indirectly reduce membrane permeability for Ca ions due to its inhibiting effect on these receptors.

Thus, *in vitro* troventol in a concentration of 10^{-3} mg/ml reduces membrane permeability for Ca ions and inhibits histamine secretion by peritoneal exudate cells. Troventol affinity for M-cholinoreceptors presumably surpassed that of the reference preparations.

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