INHIBITION OF ANAPHYLACTIC REACTIONS *in vitro* AND *in vivo* BY 1-METHYL-3-ISOBUTYLXANTHINE

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The antiphosphodiesterase preparation 1-methyl-3-isobutylxanthine (MIBX) inhibited anaphylactic reactions *in vitro* and *in vivo*: It inhibited the anaphylactic liberation of histamine from isolated rat mast cells, the anaphylactic contraction of the isolated guinea pig trachea, the passive cutaneous anaphylactic reaction (PCAR) in mice, and anaphylactic bronchospasm (AB) in guinea pigs. The MIBX inhibited the anaphylactic reaction of the trachea, PCAR, and AB to a greater degree than the corresponding reactions induced by histamine.

KEY WORDS: *anaphylactic reaction; phosphodiesterase inhibitor.*

The antianaphylactic action of substances causing the accumulation of cyclic AMP in the cells is effected on target cells (mast cells) through the inhibition of liberation of the mediators of anaphylaxis from them [2, 3]. The compound 1-methyl-3-isobutylxanthine (MIBX) has proved to be a very active inhibitor of phosphodiesterase; it caused a marked increase in the cyclic AMP content in mast cells [2, 5] and inhibited the anaphylactic liberation of histamine from them [2].

For the reasons given above it was decided to compare the antianaphylactic action of MIBX on models of cellular and tissue anaphylaxis and also in experiments *in vivo*.

In this investigation the action of MIBX was studied on the anaphylactic reaction of isolated mast cells and a preparation of the isolated trachea, on the passive cutaneous anaphylactic reaction (PCAR), and on anaphylactic bronchospasm (AB).

EXPERIMENTAL METHOD

The method of reproducing the anaphylactic liberation of histamine from isolated rat mast cells was described previously [3, 4]. Female Wistar rats weighing 150-200 g were sensitized with horse serum [3].

The PCAR caused by IgE antibodies [6, 8] was reproduced by the method described previously [6, 9] in noninbred albino mice weighing 20-22 g by intravenous injection of a reacting dose of 1 mg ovalbumin in 0.5% solution of Evans' blue in a volume of 0.1 ml. The mice were given an intradermal injection of corresponding (1:2) dilutions of antiserum against ovalbumin in a volume of 30 µl 72 h before the reacting injection. The antiserum was obtained from BALB/c mice sensitized by two intraperitoneal injections (at an interval of 4 weeks) of 0.5 µg ovalbumin adsorbed on alumina [6], in a volume of 0.5 ml. The antiserum was obtained on the seventh day after the second injection of the antigen. Mixed serum from 10 mice was used. The PCAR was read 20 min after the reacting injection of the antigen. The cutaneous reaction to histamine (CRH) was assessed from the staining of the skin 10 min after intradermal injection of 1 µg histamine in a volume of 30 µl after intravenous injection of 0.2 ml of a 0.5% solution of Evans' blue. The intensity of staining of the skin was

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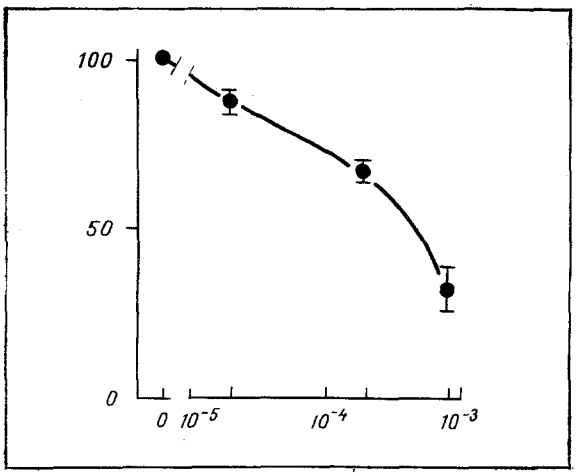

Fig. 1

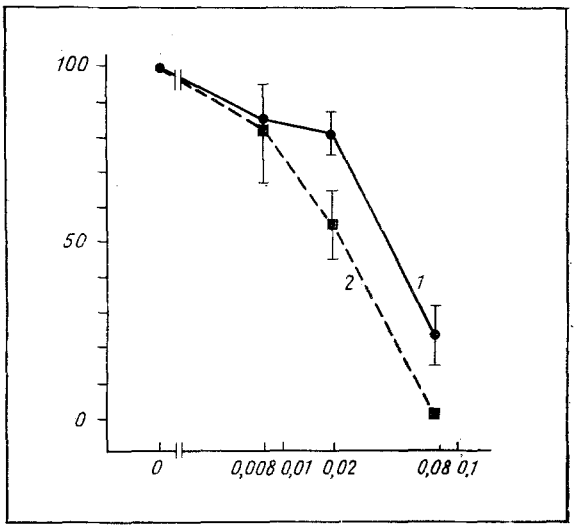

Fig. 2

Fig. 1. Action of MIBX on anaphylactic liberation of histamine from mast cells. Anaphylactic liberation of histamine in control 24.3 ± 3.8% of the total histamine content in the batch of cells. Spontaneous liberation of histamine 1.68 ± 0.12%. Cells preincubated for 15 min at 37°C in absence (control) or presence of MIBX, after which antigen (horse serum in a final concentration of $5 \cdot 10^{-3}$ liter/liter) was added. Values of M ± m shown on curve. Abscissa, concentration of MIBX (in M, logarithmic scale); ordinate, quantity of histamine liberated (in % of control).

Fig. 2. Effect of MIBX on contractions of isolated guinea pig trachea induced by histamine (1) and by antigen (2). Preincubation for 5 min. Histamine 1 μg/ml; ovalbumin 0.2 μg/ml. Degree of anaphylactic contraction in control was 69 ± 5.2% of contraction to histamine (1 μg/ml) tested before addition of antigen. Abscissa, concentration of MIBX (in mM); ordinate, degree of contraction (in % of control).

TABLE 1. Action of MIBX on PCAR and on CRH in Mice (M ± m)

	Titer (T) of PCAR $\left(\log_2 \frac{1}{T}\right)$	Intensity of reaction	
		PCAR	CRH
Control	6,25±0,32 (14)	1,985±0,24 (14)	0,855±0,137 (10)
MIBX (0.8 mg per mouse)	5,32±0,31 (14)	0,587±0,069 (14)	0,448±0,079 (10)
P	<0,05	<0,001	<0,02

Legend. Intensity of reaction expressed as amount (in μg) of Evans' blue extracted from skin. In PCAR, dye extracted from two areas of skin (in the same portion), into which serum was injected in dilutions giving a positive reaction one or two degrees of dilution lower than in titer; in CRH, from two areas of skin (in one portion), into each of which histamine was injected in a dose of 1 μg. Number of animals given in parentheses.

measured spectrophotometrically (λ = 594 nm) from the amount of dye extracted from the skin with formamide [7] at 37°C in the course of 4 days.

The method of reproducing anaphylactic contraction (under isomeric conditions) of an isolated preparation of chains of tracheal rings and of AB *in vivo* in guinea pigs was described previously [1]. Male guinea pigs weighing 300-400 g, sensitized with ovalbumin [1], were used.

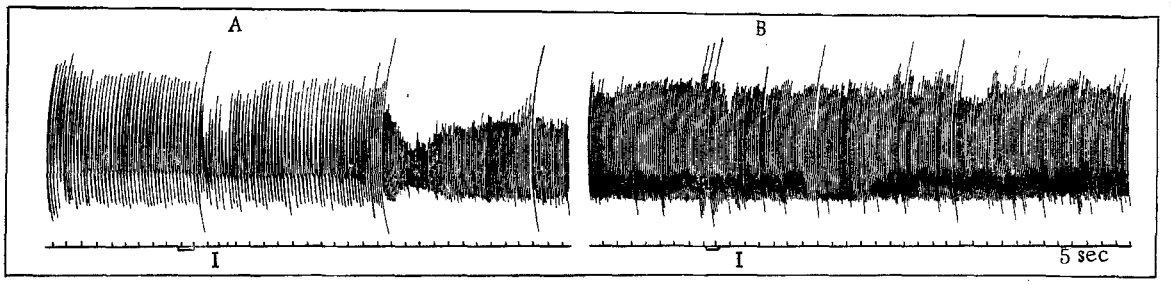

Fig. 3. Anaphylactic bronchospasm (A) after intravenous injection of ovalbumin (0.25 mg/100 g body weight) and absence of reaction (B) to injection of antigen after MIBX (0.4 mg/100 g body weight). MIBX was injected intravenously 5 min before the antigen. I) Time of injection.

The MIBX, synthesized in the Laboratory of Synthesis of Antileukemic Agents (Director, Professor R. G. Glushkov) of the S. Ordzhonikidze Scientific-Research Institute of Pharmaceutical Chemistry, was dissolved in distilled water (19 mg/ml) with minimal addition of NaOH. Working dilutions were made up from this solution.

EXPERIMENTAL RESULTS

Inhibition of the anaphylactic liberation of histamine from isolated mast cells in rats by MIBX is shown in Fig. 1 as a function of dose. In concentrations of up to 0.8 mM, MIBX itself did not cause the liberation of histamine to an amount exceeding the spontaneous level.

Between concentrations of 0.008 and 0.08 mM, MIBX caused relaxation of the isolated preparation of guinea pig trachea by an amount which depended on dose and reached a maximum of 105 ± 20.1 mg. Preincubation of the trachea for 5 min in the presence of MIBX led to dose-dependent inhibition of the contractions evoked by histamine and the antigen (Fig. 2). It will be clear from Fig. 2 that anaphylactic contraction was inhibited more than contraction produced by histamine. These differences were statistically significant for points corresponding to MIBX in concentrations of 0.02 and 0.08 mM ($P < 0.05$ and < 0.02, respectively).

Intravenous injection of MIBX in a dose of 0.8 mg per mouse 15 min before intravenous injection of the antigen inhibited the PCAR and CRH (Table 1). In this case also, the anaphylactic reaction was inhibited more (more than threefold) than the reaction to histamine (less than twofold).

Intravenous injection of MIBX into guinea pigs in a dose of 0.4 mg/100 g body weight changed neither the amplitude nor the frequency of the respiratory movements. In the same dose, MIBX had no appreciable effect on submaximal bronchospasm induced by intravenous injection of histamine in a dose of 5 µg/100 g body weight (tests on four animals).

In the same dose MIBX prevented the development of submaximal AB induced by intravenous injection of antigen in a dose of 0.25 mg/100 g body weight (Fig. 3). In control tests (five animals) AB developed in every case and the amplitude of the respiratory movements was reduced by 75 ± 12.5% after injection of the antigen. The latent period of the response was 42 ± 1.2 sec and the duration of AB was 49 ± 3.6 sec.

The results of this investigation confirmed earlier observations that the anaphylactic liberation of histamine from mast cells, which has been shown to be due to the accumulation of cyclic AMP in those cells [2], is inhibited by the antiphosphodiesterase compound MIBX.

Also, MIBX has an inhibitory action on tissue anaphylaxis, as is confirmed by the results obtained with the model of anaphylactic contraction of the isolated preparation of guinea pig trachea.

The antianaphylactic action of MIBX, moreover, is preserved in experiments *in vivo* also: MIBX significantly inhibited both the PCAR in mice and the development of AB in guinea pigs.

In all cases when tested on the trachea and on the PCAR and AB models, MIBX inhibited anaphylactic reactions more than the corresponding reactions evoked by histamine. The antianaphylactic reaction of MIBX can evidently be explained by inhibition of liberation of bio-

logically active substances from target cells (mast cells) of the allergic reaction, and by a decrease in the sensitivity of the effector tissues to the liberated mediators of anaphylaxis and, in particular, to histamine.

These findings will serve as the basis for future research aimed at conducting clinical trials of MIBX.

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ADRENERGIC AND CHOLINERGIC STRUCTURES OF THE LUNGS IN NORMAL GUINEA PIGS AND IN GUINEA PIGS WITH EXPERIMENTAL BRONCHIAL ASTHMA

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The morphological and functional state of the adrenergic and cholinergic innervation of the bronchopulmonary apparatus of guinea pigs was studied by luminescence and histochemical methods under normal conditions and during the course of sensitization (subcutaneous and inhalation methods) by the allergen of the mite *Dermatophagoides pteronyssinus*. Considerable excitation of the adrenergic innervation and a fall in acetylcholinesterase activity were observed in the bronchopulmonary tissue during sensitization of the animals.

KEY WORDS: *allergy; bronchial asthma; adrenergic system; cholinergic system.*

There is clinical evidence in the literature [4-6, 12, 14, 15] of the pathogenetic role of the allergenic component of the mite *Dermatophagoides pteronyssinus* in the development of bronchial asthma and other allergic diseases in man. It is claimed that the pathogenesis of allergic bronchial asthma is based on the hereditary or acquired blockage of the β-adrenergic receptors of the bronchopulmonary apparatus [1, 2]. The β-adrenergic theory of development of bronchial asthma is a significant addition to the allergic theory of its development.

The aim of the present investigation was to study the state of the adrenergic and cholinergic structures of the bronchopulmonary apparatus in normal guinea pigs and during the development of sensitization of the animals (produced by different methods) by the allergen of the mite *Dermatophagoides pteronyssinus*.

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