Evaluation of different anti-asthmatic drugs on cooling-induced bronchoconstriction

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Abstract

Background: Inhalation of cold air is a well-recognized cause of bronchoconstriction in asthmatics. Sudden changes in weather temperature outdoors and indoors due to the extensive use of air conditioning in Kuwait is an important existing problem.

Objectives: The aim of this study was to determine the most effective anti-asthmatic drugs for preventing or reversing cooling-induced contraction (CIC).

Methods: We recorded isometric tension from tracheal strips, and bronchiolar rings were prepared from male Merino sheep in organ baths during stepwise cooling. CIC was tested before and after addition of various standard agents. Disodium cromoglycate (DSG), methyl prednisolone, atropine, aminophylline, isoprenaline and adrenaline were examined in two cases. The first was before cooling and the second was after cooling induced the maximum bronchoconstriction.

Results: Cooling to 20°C induced a rapid and reproducible contraction in ovine tracheal and bronchial preparations. On readjustment to 37°C, the tone returned rapidly to basal level. DSG, methyl prednisolone and atropine did not prevent or reverse the CIC. Aminophylline prevented CIC by 70%. It inhibited the peak of cooling response by 70%. β-agonists (isoprenaline and adrenaline) abolished the CIC, and they also rapidly and totally reversed the cooling effect when added at the peak of bronchoconstriction.

Conclusions: These results proved that β-agonists are the drugs of choice in preventing the bronchoconstriction before exposure to cold environment and also completely reversing the bronchoconstriction induced after cooling exposure.

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Key words
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Dr. Seham Mustafa is the only author for this paper.

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I used sheep trachea and lung obtained from the slaughter house.

Conflict of interest
Author states that there is no any conflict of interest exist.

Introduction

Inhalation of cold air is a well-known cause of bronchoconstriction in asthmatics. Sudden change in weather temperature is an important existing problem. Mustafa *et al*. (1, 2) showed that cooling ovine tracheal and bronchiolar smooth muscle preparations rapidly induces contractile responses proportional to cooling temperature. Cooling-induced contraction (CIC) is due to an increase in intracellular calcium. This is brought about by release of calcium from intracellular storage sites and inhibition of calcium removal mechanisms, both calcium extrusion and sequestration (3).

Asthma is a disorder of the conducting airways that contract too easily and too much to cause variable airflow obstruction with symptoms of wheeze, cough, chest tightness and shortness of breath. Based on this knowledge, initial treatments were directed to dilating the contracted airways with anticholinergic and adrenergic drugs. The recognition that allergic-type inflammation underlay the hyperresponsive airways in asthma led to the introduction of anti-inflammatory drugs such as sodium cromoglycate and corticosteroids (4).

It is generally held that relaxation of bronchial smooth muscle is the most important anti-asthmatic effect of β-adrenoceptor stimulants as well as of

methyxanthines (5). Disodium cromoglycate (DSG) proved having a direct action on bronchial smooth muscle in addition to the inhibition of the release of chemical mediators from mast cells (6). Glucocorticoids are the most potent weapon in the treatment of asthma. Glucocorticoids favorably influence the most important pathogenetic factors in asthmatic disease, bronchoconstriction, mucosal edema and production of mucus (7). The aim of this study was to determine the most effective bronchodilator of these drugs for preventing the occurrence or reversing the effect of CIC, which is common in Kuwait.

Materials and methods

Preparation of ovine isolated tracheal strips and bronchiolar segments

The trachea together with the lung of 20 Merino sheep were obtained from the slaughter house, placed in chilled Krebs' solution and transported to the laboratory within 30 min. A piece of the trachea was cleaned of adhering adipose and connective tissue, and opened longitudinally through the cartilage rings diametrically opposite the trachealis muscle. Thereafter, it was pinned flat on a cork board, and strips of smooth muscle 10 mm in length and 5 mm in width were dissected free from the underlying cartilage. An incision was made in the parenchyma, and small bronchioles (3–4 mm in diameter) were dissected out without damage to the epithelium and cut into 5 mm ring segments. Preparations were suspended in 10-mL organ baths containing Krebs' solution, in mM: NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2, NaH_2PO_4 1.2, $NaHCO_3$ 26, glucose 11.1, at pH 7.4, maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Tension was recorded using computerized, fully automated isometric transducers (Schuler organ bath type 809; Hugo Sachs Electronik, March-Hugstetten, Germany) connected to a Gould recorder. Tracheal strips were suspended at the optimal tension of 2 g and bronchiolar rings to 1 g. The tracheal and bronchial preparations were allowed to equilibrate for 60 min, during which time they were washed twice before adding drugs.

Cooling protocol

In this study, cooling to 20°C produced a submaximal contraction, corresponding to approximately 30% of the maximum response (which was obtained at 5°C) (1). This submaximal temperature was used throughout the present investigation. The bath temperature was reduced by using a cooling circulator (Haake F3 Fisons, Germany), which had been set to 20°C. It took 2–3 min to reach the desired temperature (from 37°C to 20°C). The cooling period was maintained until a peak response was obtained. Different anti-asthmatic drugs were used for two series of experiments to examine their effects on CIC. In the first method, the anti-asthmatic drugs were added when cooling induced its bronchoconstriction maximum effect. While in the second method, the bronchodilators were added 30 min before cooling the ovine airways. Responses were calculated as mg/mg tissue weight.

Drugs

These included carbachol hydrochloride, atropine sulphate, DSG, aminophylline, isoprenaline hydrochloride, adrenaline and methyl prednisolone (all obtained from Sigma, St. Louis, MO, USA). All drugs were dissolved in distilled water.

Calculation

Data are presented as mean ± standard error of the mean of (n) experiments. Where necessary, differences between two mean values were compared using Student's 't' test paired or unpaired as appropriate. Where multiple comparisons were necessary, one-way ANOVA was used followed by Student–Newman–Keuls test. The difference was assumed to be significant where $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$.

Results

Cooling to 20°C induced a contraction in tracheal and bronchiolar smooth muscle, which was rapid in onset and reached peak tension in about 8 min. The contractile response decreased slowly until it reached the basal level in about 30 min. If the temperature was returned to 37°C, the tone returned rapidly to the basal level in about 1 min (Fig. 1).

DSG (100 μM), aminophylline (1 mM), atropine (1 μM), β-agonists (isoprenaline and adrenaline, 10 μM) and steroids (methyl prednisolone, 1 mM) are the main drug groups used in the prophylaxis and treatment of bronchial asthma. The effects of these drugs were examined in two cases. The first was before cooling and the second was after cooling induced the maximum bronchoconstriction. The concentrations of these drugs were chosen according to the previous studies. They produced 100% dilataion on the constriction due to KCl or carbachol.

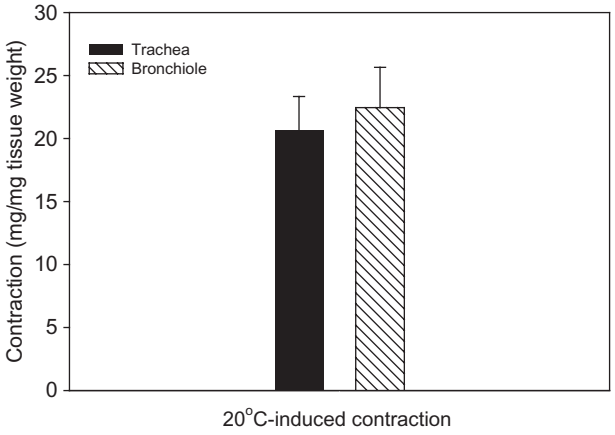

Figure 1. Effects of cooling-induced contraction on basal tone in ovine airway smooth muscle. Values are the means ± standard error of the mean (SEM) of 30 experiments.

Reversal of cooling-induced constriction

DSG, methyl prednisolone and atropine had no effect on the magnitude of the cooling response. However, aminophylline inhibited the peak of the cooling response by 70%. Isoprenaline and adrenaline rapidly returned the tone to the basal level. Both totally reversed the cooling effect for tracheal and bronchiolar smooth muscles, as shown in Fig. 2.

Effect of the anti-asthmatic drugs before cooling

DSG (100 μM), methyl prednisolone (1 mM) and atropine (1 μM), did not prevent CIC. Aminophylline (1 mM) inhibited the cooling effect by 70%, while isoprenaline and adrenaline (10 μM) (β-agonists) abolished the CIC for tracheal and bronchiolar smooth muscles, as shown in Figs. 3 and 4

Discussions

Results from previous studies (1–3) confirmed that CIC is due to an increase in intracellular calcium, which induces contraction. This is brought about by release of Ca^{2+} from intracellular storage sites and inhibition of calcium removal mechanisms, both calcium extrusion and sequestration, and decreasing the activity of the adenosine triphosphatases (ATPases). This will lead to further constriction of reflexly constricted airway smooth muscle in asthmatic patient.

There are two categories of anti-asthma drugs: bronchodilators and anti-inflammatory agents. Bronchodilators reverse the bronchospasm of the immediate phase; anti-inflammatory agents inhibit or prevent

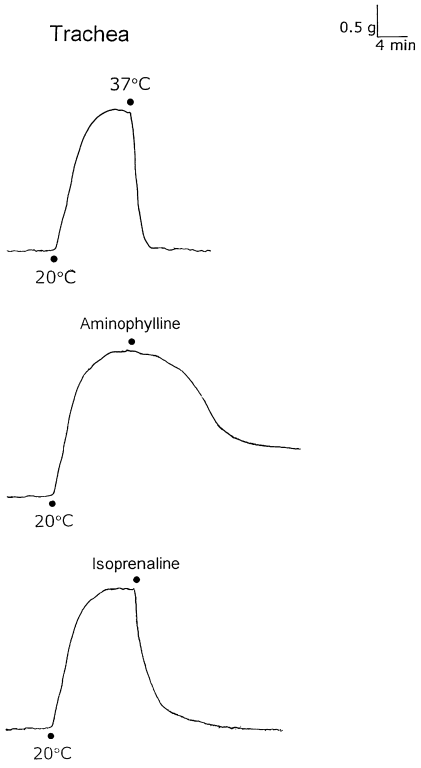

Figure 2. Original tracings of the effect of cooling temperature (20°C) on ovine trachea and its return to the basal tone at 37°C, with aminophylline (1 mM) and with isoprenaline (10 μM).

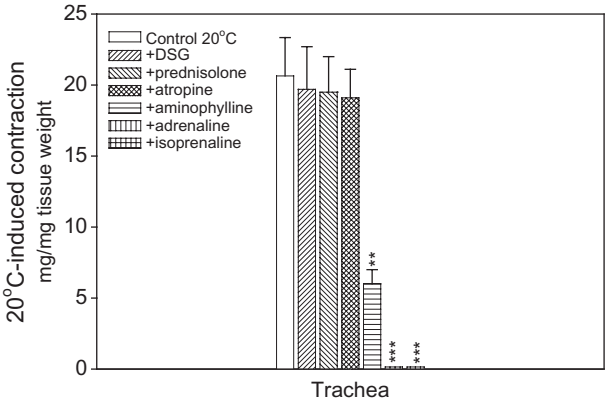

Figure 3. Effects of (A) disodium cromoglycate (100 μM), (B) methyl prednisolone (1 mM), (C) aminophylline (1 Mm), (D) atropine (1 μM), (E) isoprenaline (10 μM) and (F) adrenaline (10 μM) on cooling temperature (20°C)-induced contraction in ovine trachea. Each point represents the mean ± standard error of the mean (SEM) of 10 experiments. $^{**}P < 0.005$, $^{***}P < 0.001$.

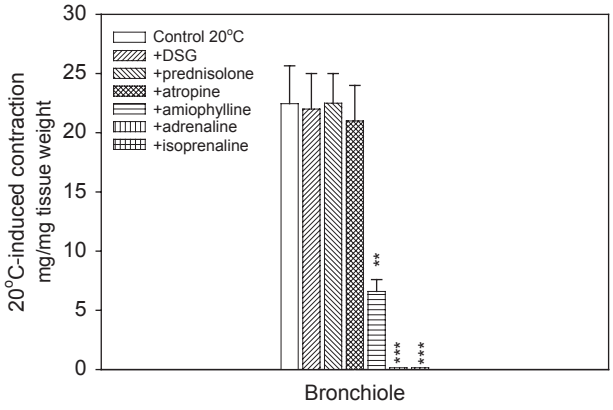

Figure 4. Effects of (A) disodium cromoglycate (100 μM), (B) methyl prednisolone (1 mM), (C) aminophylline (1 mM), (D) atropine (1 μM), (E) isoprenaline (10 μM) and (F) isoprenaline (10 μM) on cooling temperature (20°C)-induced contraction in ovine bronchiole. Each point represents the mean ± standard error of the mean (SEM) of 10 experiments. $^{**}P < 0.005$, $^{***}P < 0.001$.

the inflammatory components of both phases. These two categories are not mutually exclusive: some drugs classified as bronchodilators also have some anti-inflammatory effect (8). How best to use these drugs to treat asthma is complex. A guideline from the British Thoracic Society, updated in 2012 (9), specifies the therapeutic steps to treat asthma. The main drugs used as bronchodilators are β-adrenoceptor agonists; others include xanthines and muscarinic receptor antagonists (10). The β-adrenoceptors are present in sheep trachea (11). The β-adrenoceptor agonists dilate the respiratory system by a direct action on the β-adrenoceptors on the smooth muscle. They may act by increasing the intracellular level of cyclic adenosine 3′,5′-monophosphate (cAMP) through stimulation of adenylate cyclase (12). They also inhibit mediator release from mast cells and tumor necrosis factor-α release from monocytes (13). In this study, isoprenaline and adrenaline abolished the effect of CIC. They also abolished the cooling effect after adding it at the peak of the cooling contraction at 20°C. cAMP was established as a true second messenger (14). There are a number of possible mechanisms whereby cAMP relaxes smooth muscle. cAMP may lower cytoplasmic Ca^{2+} concentrations by stimulating Ca^{2+} extrusion from the cell via the plasmlemmal Ca^{2+}-ATPase pump and enhancing the sequestration of Ca^{2+} into the intracellular store; in addition, cAMP may block the phosphoinositol signaling pathway for the release of intracellular Ca^{2+} and also have a direct inhibitory effect on contraction via phosphorylation of myosin light chain kinase (15). Opening the K^+-channel which repolarizes the smooth muscle cell can also occur (16).

Aminophylline is also a drug used for asthma. It inhibits phosphodiesterase and blocks adenosine receptors (17). Aminophylline prevented CIC by 70%. It also slowly inhibited the peak of cooling response by 70%. Its maximum relaxant response took double the time taken by isoprenaline.

The main drugs used for their anti-inflammatory action in asthma are the glucocorticoids. They are effective in acute severe asthma (18). An important action, of relevance for asthma, is that they decrease formation of cytokines, in particular the T helper type 2 cytokines that recruit and activate eosinophils and are responsible for promoting the production of immunoglobulin E (IgE) and the expression of IgE receptors. Glucocorticoids also inhibit the generation of the vasodilators prostaglandin E_2 and prostaglandin I_2 by inhibiting induction of cyclo-oxygenase-2. By inducing annexin 1, they could inhibit production of leukotrienes and platelet-activating factor (19). Chung and Jones (20) demonstrated the powerful bronchodilator action of DSG and its inhibitory action on exercise-induced bronchoconstriction in asthmatic children. They raised the possibility that sodium cromoglycate might have a direct action on inhibiting the release of chemical mediators of mast cells. It is suggested that DSG might attenuate the action of bronchoconstrictors and potentiate the action of bronchodilators by preventing the influx of calcium into the bronchial smooth muscle cells resulting in stabilization of the cell membrane (6). Cromoglycate is a 'mast cell stabiliser', preventing histamine release from mast cells (21).

Atropine does not discriminate between muscarinic receptor subtypes as ipratropium. It is possible that its blockade of M_2 autoreceptors on the cholinergic nerves increases acetylcholine release and reduces the effectiveness of its antagonism at the M_3 receptors on the smooth muscle.

DSG and the methyl prednisolone did not prevent or reverse the CIC. They have no effect on cooling response because of their lack of effect on intracellular calcium level, which is the main cause for CIC.

Our results proved that DSG, atropine and steroids (methyl prednisolone) had no effect on CIC. Therefore, they cannot be used to prevent the occurrence of CIC or to treat the bronchoconstriction due to cooling. Therefore, they cannot be used to prevent the occurrence of CIC or to treat the bronchoconstriction of CIC. Aminophylline had slow and moderate effect before and after CIC. However, β-agonists (isoprenaline and adrenaline) are the drugs of choice in preventing the constriction due to cooling. They also

completely reversed the constriction induced after cooling exposure. These results will not only be helpful to the countries with cold weather, but also to the countries with very hot weather due to the extensive use of air conditioning and the sudden changes in weather temperature outdoors and indoors.

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