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Inhibition of allergen-induced early reactions in atopic skin by salbutamol and theophylline

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Abstract

The possible antagonistic effect of the beta$_2$-adrenoceptor agonist salbutamol and the methylxanthine theophylline on allergen-induced immediate skin reactions was elucidated. Dose-dependent reductions of early allergen-induced responses (flare and weal) were produced in eight atopic patients by salbutamol, 2.5 ng–1 μg ($p < 0.001$). Theophylline attenuated these responses only at a high dose, 100 μg ($p < 0.01$). Histamine-induced flare responses were not influenced by these agents, but wealing was inhibited by 35% by 1 μg of salbutamol ($p < 0.001$). It is concluded that agents which interact with anaphylactic histamine release and elevate cyclic AMP level in heterogenous tissues *in vitro* have similar counteracting effects on allergen-induced skin reactions in atopic subjects.

Introduction

The beta-adrenoceptor agonist terbutaline inhibits allergen-induced skin reactions in atopic subjects and this effect is blocked by propranolol [1, 2]. Studies with various beta-agonists and antagonists have suggested that the anti-allergic action of beta-agonists is mediated through activation of beta$_2$-receptors, possibly on the mast cell, resulting in inhibition of mediator release [3]. Both beta-adrenoceptor agonists and methylxanthines inhibit mediator release in sensitized heterogeneous tissues *in vitro* concomitantly with an increase in cyclic 3′,5′-adenosine monophosphate (cyclic AMP) contents of the tissue. Conversely, agents which decrease cyclic AMP and/or increase cyclic GMP levels, such as alpha-adrenoceptor agonists, enhance the mediator release [4]. The aim of the present study was to further evaluate the effects of agents known to influence cyclic AMP levels in various tissues on allergen-induced reactions in atopic skin. The agents thus studied were salbutamol and theophylline.

Patients and methods

Eight atopic subjects (3 females, 5 males) aged 20–32 years (mean 23.4) devoid of dermographism or atopic dermatitis and with a history of moderate extrinsic rhinitis and/or bronchial asthma as well as a positive immediate skin test were selected for the study. Most of the patients had RAST scores of ≥2 to horse dander allergen. The patients were allowed to use aerosols of beta$_2$-agonists but deprived of any oral medication during at least 10 days before skin testing and gave their informed consent to participate in the study which had been approved by the local Ethical Committee.

Skin testing

The patients' back was divided into 20 sections from the upper level of the scapules to the second lumbar vertebra giving four injection sites at each level. Incremental concentrations of histamine (0.67 ng–2 μg) in 0.02 ml or horse dander allergen (w/v) were injected intradermally (i.d.) in a pretrial test and the extents of the flare and weal responses at 15 min were outlined on a transparent film. The respective areas were calculated by planimetry. Dose–response curves were constructed from these results and the ED_{50} for the histamine-induced flare response was determined from the curve and a comparable allergen dose was subsequently selected in each individual. On a second occasion skin sites were treated in a random fashion with 0.02 ml of the trial drugs in selected doses or vehicle (control) followed by injection of allergen or histamine delivered by a micrometer syringe 5 min later. This treatment schedule was based on a previous finding [1] that terbutaline proved to be more effective as an inhibitory drug when administered i.d. shortly before rather than simultaneously with the allergen. Theophylline, on the other hand, produced analogous inhibition in this respect in a few subjects in a pretrial test. Administration of the drugs used produced no obscuring effects in the selected dose range, i.e. no obvious vascular effects (flare and weal), thereby permitting the evaluation of anti-allergic and anti-histaminic properties of the agents. Student's *t*-test for paired observations was employed for statistical within-subject analysis of responses at drug-treated sites vs those of simultaneously injected control sites at each injection level.

Chemicals

Salbutamol sulphate (Ventoline solution®, 0.5 mg/ml, Glaxo, Mölndal, Sweden) a beta$_2$-selective agonist and theophylline (Teofyllamin®, 23 mg/ml, theophylline and ethylendiamine according to the Europ Pharmacopoeia, ACO AB, Solna, Sweden) a methylxanthine derivative, were

dissolved in physiological saline containing 10% v/v Sorensen phosphate buffer (Na_2HPO_4 + KH_2PO_4, 67 m*M*) pH 7.4. An aqueous extract of horse dander allergen (Vitrum®, 10^{-1} w/v, Stockholm, Sweden) or histamine dihydrochloride (1 mg/ml) were further diluted in physiological saline containing 0.3% phenol.

Results

Salbutamol solutions of 5.2×10^{-7}–2.1×10^{-4} *M* (2.5 ng–1 μg), produced a dose-dependent inhibition of the allergen-induced flare response, whereas a maximal anti-weal effect was accomplished already in the lower concentration range of the drug in five subjects ($p < 0.001$, Fig. 1). These anti-allergic effects were antagonized by propranolol (not shown). Theophylline, 5.5×10^{-4}–2.2×10^{-2} *M* solutions (2.5–100 μg), induced a less clear-cut antagonism of the responses to allergen only significant when injected in the maximal concentration ($p < 0.01$, Fig. 1). Effects of the drugs, injected in maximal doses, on responses to allergen and histamine are presented in Table 1. The flare response to histamine was not influenced by salbutamol or theophylline but the wealing was reduced 35% by 1 μg of salbutamol ($p < 0.001$).

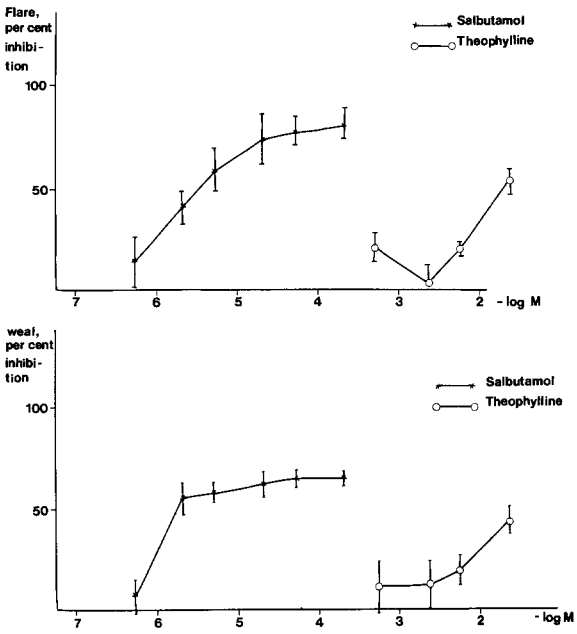

Figure 1
Dose–response relation of the effect, expressed as per cent inhibition, of salbutamol and theophylline (–log *M* concentration injected) on the allergen-induced flare (a) and weal (b) response measured at 15 min. The compounds were injected intradermally 5 min prior to allergen (mean ± SEM; $n = 5$).

The concentrations of allergen needed to produce flare responses of a size similar to that elicited by the ED_{50} for histamine varied between 10^{-10}–10^{-4} w/v due to differences in sensitivity of the allergic subjects. The coefficient of variation for reactions to allergen ($n = 8$) injected simultaneously at two sites at the same level was 12% (flare) and 20% (weal).

Discussion

The beta$_2$-adrenoceptor agonist salbutamol and the methylxanthine theophylline antagonized allergen-induced reactions in atopic skin in agreement with previous results concerning inhibition of histamine release from passively sensitized human lung fragments [5, 6]. Since the compounds had no (theophylline) or limited (salbutamol) effect on the responses to histamine the suppression of reactions to allergen appears to be caused by inhibition of mediator release, presumably from mast cells. This concept was further supported by the findings that skin sites antigen-challenged and treated with terbutaline

Table 1
Effect of salbutamol and theophylline, injected intradermally in the maximal dosages, on the flare and weal responses (mm^2) to allergen and histamine (mean ± SEM; $n = 8$).

	Dosage of drug (μg)	Allergen				Histamine			
		Weal		Flare		Weal		Flare	
		Drug	Control	Drug	Control	Drug	Control	Drug	Control
Salbutamol	1	25 ± 2***	75 ± 9	230 ± 61***	1215 ± 39	55 ± 7***	80 ± 6	1055 ± 109	1080 ± 106
Theophylline	100	40 ± 5**	80 ± 8	540 ± 65**	1175 ± 96	85 ± 12	80 ± 6	1125 ± 128	1120 ± 131

, * = significantly inhibited compared with control (evaluated by Student's paired *t*-test) $p < 0.01$ and $p < 0.001$ respectively.
The drugs were injected 5 min prior to the challenging agents and the areas of flare and weal responses were measured at 15 min.

revealed no ultramicroscopic alteration of the mast cells [2] and gave a full response on rechallenge with another allergen 24 h later [7], as compared to conditions at non-protected allergen-challenged control sites. Previous reports of a dose-dependent antagonism of allergen-induced reactions by terbutaline and fenoterol [3, 8] are supported by the present data on salbutamol and strengthen the view that activation of $beta_2$-adrenoceptors is involved. $Beta_2$-agonists do not influence the flare response to histamine but do attenuate the wealing indicating an additional anti-inflammatory effect through antagonism of permeability increase induced by released mediators [9, 10]. Both of these effects, i.e. inhibition of mediator release and permeability changes, are, as expected, counteracted by propranolol [3, 9] and might be of therapeutic interest in allergic asthma and add to the bronchodilator capacity of the drugs.

In contrast to the demonstrated suppression of allergen-elicited cutaneous reactions by topically applied drugs, therapeutically relevant concentrations of terbutaline, theophylline and their combination lack attenuating capacity in this respect [11, 12]. It is conceivable that the demonstrated anti-allergic effect of theophylline and salbutamol was accomplished due to a more favourable relation between concentration of allergen and drugs at the challenged skin site. Theophylline exerted a significant inhibitory effect at an injected concentration above 10^{-3} *M*. This effect may have been mediated by elevation of cyclic AMP levels via phosphodiesterase antagonism, a mechanism of action described in several tissues, including mast cells, in high concentrations of the drug [13, 14]. Furthermore, i.d. injection of dibutyryl cyclic AMP suppressed the wealing to allergen in atopics [15]. Conversely, strong evidence was presented [14, 16] against a central role of cyclic nucleotides in the regulation of anaphylactic histamine release assessed by isolated rat mast cells and lower concentrations of methylxanthines ($<10^4$ *M*), instead antagonism of effects of endogenous adenosine was proposed. Another cell type adjacent to mast cells was suggested [14] as the target for cyclic nucleotide-directed actions in sensitized heterogeneous tissues and this indirect effect or mast cell heterogeneity might account for the consistent findings of inhibition of mast cell mediator release in human skin by local therapy with $beta_2$-agonists.

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