# The ability of salbutamol and theophylline to suppress immediate allergic conjunctivitis in the guinea pig

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**Abstract.** Topically administered salbutamol was extremely effective in suppressing immediate allergic conjunctivitis in the guinea pig; a dose as low as 0.1% elicited 98% inhibition. Topical pretreatment with 1% propranolol completely blocked the suppressant action of 0.1% salbutamol. This was also the case after systemic propranolol (1 mg/kg SC); the beta-adrenoceptor antagonist itself has no effect on antigen-induced inflammation. The effect of 0.1% salbutamol was unaltered by pretreatment with the specific $beta_1$-adrenoceptor antagonist betaxolol (1 mg/kg SC). In marked contrast, the suppressant action of 0.1% salbutamol was profoundly inhibited by pretreatment with the selective $beta_2$-adrenoceptor antagonist ICI-118,551 (0.5 mg/kg SC). The experiments employing beta-adrenoceptor antagonists unequivocally demonstrate that the salbutamol suppression of immediate allergic conjunctivitis in the guinea pig is mediated via the activation of $beta_2$-adrenoceptors. The methylxanthine phosphodiesterase inhibitor theophylline was active after oral administration, 50 mg/kg eliciting an 80% inhibition. Theophylline was inactive topically at 1% and 5%, but this could be due to the fact that the compound was insoluble at these concentrations. Thus, procedures that elevate cyclic-AMP levels suppress immediate hypersensitivity reactions in the guinea pig conjunctiva. Whether or nor this offers an alternative approach to treat allergic conjunctivitis in humans remains to be determined.

## Introduction

A characteristic feature of immediate hypersensitivity is edema formation, which results from increased microvascular permeability to plasma proteins. Conjunctival edema can be elicited in sensitized guinea pigs by the local application of antigen (Dwyer and Darougar 1971). Antigen-induced edema in the guinea pig conjunctiva can be blocked by locally applied histamine $H_1$-receptor antagonists (Dwyer et al. 1976), although it has recently been reported that this response in the guinea pig consists of a histaminic and a nonhistaminic component (Woodward et al. 1986).

Immediate hypersensitivity is dependent upon the release of mediators from IgE-sensitized mast cells, and

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cyclic-AMP is considered to play a pivotal role in this process (Jackson and Gilmore 1981; Lewis and Austen 1981). Levels of cyclic-AMP are increased by beta-adrenoceptor agonists and these compounds, in addition to relaxing bronchial smooth muscle, inhibit the antigen-induced release of mediators of bronchoconstriction from sensitized guinea pig and human lung, an effect blocked by the nonselective beta-adrenoceptor antagonist propranolol (Assem and Schild 1971; Orange et al. 1971; Butchers et al. 1979; Marone et al. 1984). The use of selective beta-adrenoceptor antagonists has shown that the beta-adrenoceptor in both human and guinea pig lung, which is implicated in the immunological release of mediators, belongs to the $beta_2$-subtype (Butchers et al. 1980; Hughes et al. 1983; Undem and Buckner 1984).

The objective of this study was to determine if the topical administration of the beta-adrenoceptor agonist salbutamol could counteract an immediate hypersensitivity reaction in the guinea pig conjunctiva. This was found to be the case, and by using specific beta-adrenoceptor antagonists it was found that the beta-adrenoceptor mediating anti-anaphylactic activity belongs to the $beta_2$-subtype.

## Materials and methods

Female Dunkin-Hartley guinea pigs were used that weighed 310–520 g at the time of sensitization. The animals were sensitized by two intradermal injections in the neck region of a 0.1 ml saline solution (0.9% NaCl) containing 1 mg ovalbumin and 2 mg aluminum hydroxide. At the same time, 1 ml Bordetella pertussis vaccine ($5 \times 10^9$ killed organisms) was injected intraperitoneally.

Antigen challenge occurred after a resting period of 14 to 21 days. Preliminary experiments indicated that the response was uniform during this time period. Topical challenge consisted of three instillations (10 µl) at 10-min intervals of a 2.5% ovalbumin solution in saline. Edema of the upper and the lower conjunctiva was assessed visually, under blind conditions, 10 min after the last challenge using a scale of 0 to 3, which included half scores (Dwyer et al. 1974). Preliminary experiments indicated that the response was maximal at this time point, an observation in agreement with that of others (Dwyer and Darougar 1971).

In most experiments, Evans' blue content of the upper conjunctiva was used as a marker for edema. A 20 mg/kg solution in saline was injected into the marginal ear vein just prior to the first instillation of antigen. Animals were

**Table 1.** Effect of topically applied salbutamol on antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score |
|---|---|
| Vehicle | $1.81 \pm 0.20$ |
| Salbutamol, 0.0001% | $1.31 \pm 0.15$ |
| Salbutamol, 0.001% | $0.68 \pm 0.19$[b] |
| Salbutamol, 0.01% | $0.28 \pm 0.11$[b] |
| Salbutamol, 0.1% | $0.03 \pm 0.03$[b] |

[a] Previously sensitized guinea pigs were challenged by three instillations (10 µl) at 10-min intervals of a 2.5% ovalbumin solution. Edema was assessed visually using a scale of 0 to 3, which included half scores 10 min after the last challenge. Salbutamol (10 µl) was instilled 3 min prior to antigen challenge. Results are expressed as means $\pm$ SEM and each group consisted of 14–16 observations
[b] Significantly different from vehicle-treated, $P < 0.05$ (Mann-Whitney "$U$" test)

**Table 2.** Effect of propranolol on the ability of salbutamol to block antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score |
|---|---|
| Vehicle | $1.83 \pm 0.19$ |
| Salbutamol | $0.35 \pm 0.12$[b] |
| Propranolol + salbutamol | $1.95 + 0.19$ |

[a] Propranolol (1%) was instilled (10 µl) 30 min prior to 0.1% salbutamol. For further experimental details, see Table 1. Results are expressed as mean $\pm$ SEM and each group consisted of ten observations
[b] Significantly different from vehicle-treated, $P < 0.05$ (Mann-Whitney "$U$" test)

**Table 3.** Effect of systemically administered propranolol on the ability of topical salbutamol to block antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score | Evans' blue content (µg) |
|---|---|---|
| Vehicle | $0.95 \pm 0.13$ | $13.1 \pm 1.6$ |
| Salbutamol | $0.22 \pm 0.05$[b] | $5.9 \pm 1.0$[c] |
| Propranolol + salbutamol | $0.80 \pm 0.11$ | $11.8 \pm 1.3$ |

[a] Propranolol (1 mg/kg) was injected SC 30 min prior to salbutamol (0.1%). For further experimental details, see Table 1. Results are expressed as mean $\pm$ SEM and each group consisted of 24 observations
[b] Significantly different from vehicle-treated, $P < 0.05$ (Mann-Whitney "$U$" test)
[c] Significantly different from vehicle-treated, $P < 0.05$ (Student's $t$-test)

**Table 4.** Effect of systemically administered betaxolol or ICI-118,551 on the ability of topical salbutamol to block antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score | Evans' blue content (µg) |
|---|---|---|
| Vehicle | $1.28 \pm 0.20$ | $12.8 \pm 2.1$ |
| Salbutamol | $0.20 \pm 0.06$[b] | $3.5 \pm 0.8$[c] |
| Betaxolol + salbutamol | $0.19 \pm 0.04$[b] | $3.1 \pm 0.5$[c] |
| Vehicle | $0.88 \pm 0.14$ | $12.9 \pm 2.0$ |
| Salbutamol | $0.30 \pm 0.05$[b] | $5.3 \pm 1.1$[c] |
| ICI-118,551 + salbutamol | $1.02 \pm 0.20$ | $13.1 \pm 2.1$ |

[a] Betaxolol (1 mg/kg) or ICI-118,551 (0.5 mg/kg) was injected SC 30 min prior to topically applied salbutamol (0.1%). For further experimental details, see Table 1. Results are expressed as mean $\pm$ SEM and each group consisted of 16–20 observations
[b] Significantly different from vehicle-treated, $P < 0.05$ (Mann-Whitney "$U$" test)
[c] Significantly different from vehicle-treated, $P < 0.05$ (Student's $t$-test)

killed just after edema scoring. The upper conjunctiva was dissected, weighed, and stored frozen until dye extraction. This was achieved by using a 0.5% aqueous solution of sodium sulphate in acetone (30:70; v/v), 4 ml/100 mg tissue (Harada et al. 1971), and by placing the material in a sonicator for 30 min. Evans' blue levels were measured spectrophotometrically at 620 nm and expressed as micrograms dye per site of bluing. Results were expressed as the increase in Evans' blue content from the mean value ($1.54 \pm 0.21$ µg, $n = 14$) obtained for nonchallenged guinea pigs.

Topically administered drugs (10 µl, 1 drop) were either dissolved (D,L-propranolol hydrochloride, salbutamol sulphate) or suspended (theophylline) in 0.5% aqueous hydroxyethylcellulose (HEC). For systemic routes of administration, compounds (propranolol, betaxolol hydrochloride, ICI-118,551 hydrochloride) were dissolved in saline. Theophylline was suspended in 0.5% aqueous methylcellulose. The administration times are indicated in the results. Doses, where appropriate, refer to the free base.

Controls received appropriate vehicles and statistical differences between control and treated groups were analyzed using the nonparametric Mann-Whitney $U$ test for edema scores and Student's $t$-test for Evans' blue values.

## Results

Topical administration of doses of salbutamol, ranging from 0.0001% to 0.1% 3 min prior to antigen challenge, elicited a dose-dependent inhibition of conjunctival edema (Table 1). The essentially complete antagonism after instillation of a 0.1% solution should be noted.

A 30-min pretreatment with 1% propranolol completely attenuated the ability of a 0.1% solution of salbutamol to prevent antigen-induced conjunctival edema (Table 2). The SC injection of propranolol (1 mg/kg) 30 min prior to the instillation of 0.1% salbutamol also completely blocked the effect of the latter, as assessed in terms of conjunctival content of Evans' blue and edema score (Table 3). A 30-min pretreatment with propranolol (1 mg/kg SC) had no effect on antigen-induced anaphylaxis, as indicated by edema scores of $1.94 \pm 0.19$ and $1.90 \pm 0.15$ for vehicle- and drug-treated groups ($n = 12$), respectively. The corresponding conjunctival Evans' blue levels were $20.2 \pm 3.2$ and $17.9 \pm 2.3$ µg. The ability of 0.1% salbutamol to blunt conjunctival edema induced by antigen challenge was totally unaltered by 30-min pretreatment with the selective beta$_1$-adrenoceptor antagonist betaxolol (1 mg/kg SC) (Table 4). In contrast, pretreatment with the selective beta$_2$-adrenoceptor antagonist ICI-118,551 (0.5 mg/kg SC) was effective in attenuating the ability of topical 0.1% salbutamol, to block antigen-induced conjunctival edema.

**Table 5.** Effect of orally administered theophylline on antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score |
|---|---|
| Vehicle | $1.66 \pm 0.19$ |
| Theophylline, 10 mg/kg | $1.18 \pm 0.25$ |
| Theophylline, 20 mg/kg | $1.06 \pm 0.09$[b] |
| Theophylline, 50 mg/kg | $0.34 \pm 0.06$[b] |
| Theophylline, 100 mg/kg | $0.19 \pm 0.08$[b] |

[a] Suspensions of theophylline in 0.5% methylcellulose were administered orally 2 h before antigen challenge (see Table 1 for details). Results are expressed as mean $\pm$ SEM and each group consisted of 14-16 observations
[b] Significantly different from vehicle-treated, $P < 0.05$ (Mann-Whitney " $U$ " test)

**Table 6.** Effect of propranolol on the ability of theophylline to block antigen-induced conjunctival edema in the guinea pig[a]

| Treatment | Edema score | Evans' blue content (µg) |
|---|---|---|
| Vehicle | $1.60 \pm 0.13$ | $17.7 \pm 1.6$ |
| Theophylline, 30 mg/kg | $0.60 \pm 0.07$ | $8.9 \pm 1.5$ |
| Propranolol + theophylline | $1.03 \pm 0.18$[b] | $10.9 \pm 2.2$ |
| Vehicle | $1.31 \pm 0.15$ | $21.2 \pm 2.7$ |
| Theophylline, 100 mg/kg | $0.09 \pm 0.03$ | $2.4 \pm 0.6$ |
| Propranolol + theophylline | $0.33 \pm 0.09$[b] | $5.6 \pm 1.1$[c] |

[a] Propranolol (1 mg/kg SC) was injected 30 min prior to orally administered theophylline. Antigen challenge occurred 1 h after theophylline administration. For further experimental details, see Table 1. Results are expressed as mean $\pm$ SEM and each group consisted of 16-24 observations
[b] Significantly different from theophylline-treated, $P < 0.05$ (Mann-Whitney " $U$ " test)
[c] Significantly different from theophylline-treated, $P < 0.05$ (Student's $t$-test)

Because theophylline was in suspension, the time of pretreatment in topical studies was increased to 30 min. The instillation of 1% and 5% suspensions of theophylline was totally devoid of effect on antigen-induced conjunctival edema (data not shown). In contrast, the oral administration of doses ranging from 20 to 100 mg/kg at 2 h before challenge elicited a dose-dependent inhibition (Table 5). The effectiveness of theophylline (100 mg/kg PO) was retained when the pretreatment time was decreased from 2 to 1 h (Table 6). A 30-min pretreatment with propranolol (1 mg/kg SC) had a slight, but significant antagonistic effect on the action of both 30 and 100 mg/kg of theophylline at 1 h.

**Discussion**

The results of the present study indicate that topically administered salbutamol is very effective in blunting conjunctival edema following local antigen challenge to previously sensitized guinea pigs; a dose as low as 0.001% elicited significant antagonism. Almost total inhibition of inflammation was achieved when the instilled dose was raised to 0.1%, the amount administered being 10 µg. Others have previously shown that topically applied salbutamol prevents conjunctival inflammation in the guinea pig (Wood-

ward and Nieves 1985). However, only one dose of salbutamol (1%) was evaluated and this concentration is well in excess of those shown to be active in the present study. The beta-adrenoceptor agonist terbutaline has also been found to suppress immediate allergic conjunctivitis in the guinea pig (Linstone and Mondino 1984; Woodward and Nieves 1985) and dipivalyl epinephrine found to inhibit passive anaphylactic reaction in rat conjunctiva (Iso et al. 1980b). Characterization of the beta-adrenoceptor involved in the response by the use of specific beta-adrenoceptor antagonists has not been done and this was the objective of the present study.

The ability of a 0.1% solution of salbutamol to block antigen-induced conjunctival inflammation was totally blocked by both topical (1%) and systemic pretreatment (1 mg/kg SC) with the nonspecific beta-adrenoceptor antagonist propranolol, as assessed by visual rating of the edema and conjunctival Evans' blue levels. The latter can be viewed as giving a more accurate reflection of the anaphylactic response (Iso et al. 1980a). Systemically administered propranolol (1 mg/kg SC) was devoid of effect on antigen-induced conjunctival edema. This not only indicates that propranolol's ability to block the anti-inflammatory action of salbutamol cannot be ascribed to a nonspecific effect, but also that the activity of salbutamol in this model is clearly dependent upon the activation of beta-adrenoceptors. The two beta-adrenoceptor antagonists used for receptor classification were betaxolol and ICI-118,551. Betaxolol possesses a selectivity for $beta_1$-adrenoceptors, as indicated by $pA_2$ values of 8.76 and 6.98 for $beta_1$ (atria) and $beta_2$-adrenoceptors (trachea) in the guinea pig, respectively. The corresponding values for propranolol were 8.76 and 8.51 (Schmitt et al. 1984). The reverse applies to ICI-118,551, its $pA_2$ values for guinea pig $beta_1$- (atria) and $beta_2$-adrenoceptors (uterus) being 7.17 and 9.26, respectively. Respective values for propranolol were 8.30 and 8.64 (Bilski et al. 1983). The SC injection of 1 mg/kg betaxolol 30 min prior to topical salbutamol had no effect on the ability of the latter to suppress antigen-induced edema. It should be recalled that an identical pretreatment with propranolol was extremely effective in blocking the effect of salbutamol. Since propranolol and betaxolol possess comparable affinities for $beta_1$-adrenoceptors, the ineffectiveness of the latter strongly suggests that the action of the former is mediated via blockade of $beta_2$-adrenoceptors. Confirmation for this comes from the experiment employing ICI-118,551; the SC injection of 0.5 mg/kg of this selective $beta_2$-adrenoceptor antagonist markedly attenuated the effect of topical salbutamol. This action of ICI-118,551 cannot be attributed to an effect on $beta_1$-adrenoceptors because the affinity of the compound for these receptors is less than that of betaxolol and the latter was devoid of effect at 1 mg/kg. The experiments using beta-adrenoceptor antagonists unequivocally demonstrate that the ability of salbutamol to prevent antigen-induced anaphylaxis in the guinea pig conjunctiva is mediated via the activation of $beta_2$-adrenoceptors.

Although it is well established that the stimulation of adenylate cyclase prevents the antigen-induced release of inflammatory mediators (see Introduction), such a mechanism of action may not account totally for the observed ability of salbutamol to blunt immediate allergic conjunctivitis because the histamine-induced increase in microvascular permeability in the guinea pig conjunctiva is blocked by locally applied salbutamol (Woodward and Nieves

1985). Thus, a functional antagonism may contribute to the observed effect.

An alternative procedure to increase cyclic-AMP levels, other than by receptor activation, is to block phosphodiesterase, and experiments were undertaken using the methylxanthine theophylline. Methylxanthines such as theophylline have been previously shown to inhibit the antigen-induced release of mediators from animal and human tissues (Lichtenstein and Margolis 1968; Koopman et al. 1970; Toll and Andersson 1984). In contrast to salbutamol, topical theophylline failed to attenuate antigen-induced conjunctival edema. Perhaps this is due to the fact that the compound was administered as a suspension and that a slow dissolution rate prevented the attainment of an adequate drug concentration in the tissue for activity. Orally administered theophylline was effective, a 50 mg/kg dose eliciting an 80% inhibition of antigen-induced conjunctival edema. A 30-min pretreatment with propranolol (1 mg/kg SC) elicited a slight, but significant attenuation of the effect of both 30 mg/kg and 100 mg/kg of theophylline. However, this effect of propranolol bore no resemblance to its ability to completely blunt the action of topical salbutamol.

In summary, the results of this study clearly indicate that the ability of topical salbutamol to block antigen-induced conjunctival edema in the guinea pig is due to the activation of beta$_2$-adrenoceptors, which triggers cyclic-AMP production by adenylate cyclase. Systemic administration of the phosphodiesterase inhibitor theophylline also prevented the inflammatory response following antigen challenge. Thus, it is apparent that procedures that elevate cyclic-AMP levels prevent immediate hypersensitivity reactions in the guinea pig conjunctiva. Whether or not this offers an alternative approach to treat allergic conjunctivitis in humans remains to be determined.

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