

Rapid corticosteroid effect on β_2 -adrenergic airway and airway vascular reactivity in patients with mild asthma

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Background: Long-term glucocorticoid therapy has been suggested to improve airway and airway vascular smooth muscle responsiveness to inhaled β_2 -agonists in patients with asthma.

Objective: We sought to assess whether a single dose of an inhaled glucocorticoid acutely potentiates β_2 -adrenergic airway and airway vascular smooth muscle reactivity in asthma.

Methods: In 10 asthmatic and 10 healthy subjects, airway blood flow and FEV₁ were measured before and 30 minutes after fluticasone or placebo inhalation and 15 minutes after the subsequent inhalation of racemic albuterol (0.6 mg or 1.25 mg) or (R)-albuterol (0.3 mg or 0.6 mg).

Results: In healthy subjects all albuterol formulations increased airway blood flow equally after placebo or fluticasone pretreatment. In asthmatic subjects airway blood flow response was blunted after placebo and acutely restored after fluticasone pretreatment. Fluticasone pretreatment did not increase FEV₁ responses to any albuterol formulation, except 0.6 mg racemic albuterol.

Conclusion: A single dose of an inhaled glucocorticoid restores β_2 -adrenergic airway vasodilator responses in patients with mild asthma. The mechanism of this rapid glucocorticoid effect remains to be clarified.

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Key words: Asthma, airway blood flow, corticosteroids, fluticasone, nongenomic action, β_2 -agonists, albuterol, levalbuterol

Inhaled glucocorticoids control clinical symptoms of asthma, primarily by suppressing airway inflammation. In addition to the anti-inflammatory effects, there is emerging evidence of interactions between glucocorticoids and β_2 -agonists by which long-term glucocorticoid therapy could improve β_2 -adrenergic bronchodilation.^{1,2} In keeping with the observations on airway smooth muscle, we have shown that glucocorticoid therapy can also restore β_2 -adrenergic responsiveness of airway blood vessels derived from the bronchial circulation in patients with asthma.³

Abbreviation used

Q_{aw}: Airway blood flow

However, the molecular mechanisms by which glucocorticoids improve β_2 -adrenergic airway and vascular responsiveness remain to be fully elucidated.

Inhaled glucocorticoids have been shown to facilitate β_2 -adrenergic actions by increasing β_2 -receptor numbers⁴ and restoring β_2 -receptor coupling to G proteins that mediate adenylyl cyclase stimulation.⁵ In addition to these delayed transcriptional actions, glucocorticoids can improve pulmonary function within seconds or minutes.⁶⁻⁸ Such rapid effects of glucocorticoids have also been demonstrated in the airway vasculature.^{9,10} These rapid actions could contribute to the clinically beneficial effects of glucocorticoids¹¹; however, the underlying cellular mechanisms are clearly incompatible with transcriptional actions.¹²⁻¹⁵

Through a rapid inhibition of cell membrane transporters for organic cations,¹⁶ glucocorticoids recently have been proposed to influence the pharmacokinetics of structurally diverse cationic drugs at the tissue and cellular level. Most inhaled β_2 -agonists are cations with a positive net charge at physiologic pH and thus likely substrates for organic cation transporters expressed in airway cells. Cationic drug transport inhibition by glucocorticoids therefore could increase β_2 -agonist concentration at adrenergic receptor sites in airway tissue, thereby potentiating (or maintaining) the β_2 -agonists' effect on airway and airway vascular smooth muscle.

Presumably by acting through this mechanism on the disposal of locally released norepinephrine, inhaled glucocorticoids indeed have been shown to cause an acute, dose-dependent vasoconstriction, as reflected by a decrease in airway blood flow (Q_{aw}; ie, systemic bronchial blood flow in the anatomic dead space).^{9,10}

In the present study we therefore wished to determine whether a low-dose inhaled glucocorticoid, which *per se* has no vasoconstrictive action,¹⁷ could potentiate β_2 -agonist-induced airway and airway vascular smooth muscle relaxation. The underlying hypothesis was that pretreatment with a single glucocorticoid dose would have no effect on Q_{aw} and FEV₁ values but would potentiate the effect of a subsequently inhaled β_2 -agonist on these parameters. The experiments were carried out in healthy and asthmatic subjects, with fluticasone as a representative glucocorticoid and both racemic and (R)-albuterol as representative β_2 -adrenergic agonists. Q_{aw}, as measured with our technique, reflects systemic blood flow to the subepithelial tissue of the airway wall within the anatomic dead space; more than 80% of bronchial blood flow is directed to this tissue.

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METHODS

Study subjects

Ten healthy lifetime nonsmokers and 10 lifetime nonsmokers with physician-diagnosed mild intermittent asthma participated in the study (Table I). At study entry, all asthmatic subjects were clinically stable, had an FEV₁ of greater than 70% of predicted value, and had not used inhaled or systemic glucocorticoids or other controller medication for at least 2 weeks. All asthmatic patients used inhaled albuterol irregularly as needed for symptom control. Subjects taking cardiovascular medications were excluded. All subjects denied having experienced an acute respiratory tract infection for at least a month before the study, and no subject had an acute respiratory tract infection during the study.

The University of Miami Institutional Review Board approved the study protocol. All subjects provided written informed consent.

Study design

Each subject made 8 visits to the research laboratory. The study took place during the morning of the visit day. The subjects were instructed to abstain from ingesting alcoholic beverages the night before and coffee or caffeinated beverages on the morning of the study day. The asthmatic subjects were asked to not use albuterol for at least 12 hours before the study.

The 8 visits were grouped into 4 visit pairs to assess the effect of 4 different β_2 -adrenergic agonist formulations with and without fluticasone pretreatment. Each visit pair consisted of 1 visit with fluticasone pretreatment and 1 visit with placebo (propellant) pretreatment in random order. The sequence of visit pairs was randomized. The study was single blind, and an attempt was made to schedule the paired visit days one after the other and to not allow more than 3 days to elapse between visit pairs. The following drug combinations were administered:

- low-dose racemic albuterol (0.6 mg) after fluticasone (110 μ g) or placebo (visit pair);
- high-dose racemic albuterol (1.25 mg) after fluticasone (110 μ g) or placebo (visit pair);
- low-dose (R)-albuterol (0.3 mg) after fluticasone (110 μ g) or placebo (visit pair); and
- high-dose (R)-albuterol (0.6 mg) after fluticasone (110 μ g) or placebo (visit pair).

A computer program was used to randomize the visit pairs and the sequence of fluticasone and placebo pretreatment within the visit pairs. Racemic and (R)-albuterol were delivered with a dosimeter (Salt Labs Dosimeter, Arvin, Calif) for exact dosing. The number of breaths was either 29 or 58 for the low dose and high dose of racemic and (R)-albuterol, respectively. Fluticasone and placebo (fluticasone vehicle) were administered through a metered-dose inhaler by using a spacer. The sequence of physiologic measurements and drug administrations were the same for all visits. Systemic blood pressure, pulse, O₂ saturation, spirometric results, and Q_{aw} values were measured before and 30 minutes after inhalation of 110 μ g of fluticasone or placebo. The subjects then inhaled one of the adrenergic agonist formulations. Fifteen minutes later, the physiologic measurements were repeated.

FEV₁ was measured with a Spirovit SP10 spirometer (Welch-Allyn, Skaneateles Falls, NY). Predicted normal values were taken from Crapo et al.¹⁸ A previously validated soluble inert gas uptake method was used to measure Q_{aw}.^{19,20} Q_{aw} values were normalized for the anatomic dead space and expressed as microliters per minute per milliliter.

Statistical analysis

Comparisons between groups were performed by using $2 \times 2 \times 4$ ANOVA, with asthmatic status, placebo versus fluticasone, and β -agonist drug/dose combination as between-group factors and the change from premedication to postmedication measurements (of Q_{aw} or FEV₁) as dependent variables. The null hypothesis was rejected when the *P* value was less than .05.

TABLE I. Demographics and baseline physiologic data

	Healthy	Asthmatic
No.	10	10
Sex	7 F/3 M	8 F/2 M
Age (y; range [average])	28-52 (38)	26-48 (40)
FEV ₁ (L)*	2.85 $\pm$ 0.81	3.20 $\pm$ 0.53
FEV ₁ (% predicted)*	88.1 $\pm$ 2.8	80.4 $\pm$ 3.8
Q _{aw} (μ L $\cdot$ min ⁻¹ $\cdot$ mL ⁻¹)*	32.5 $\pm$ 0.7	43.1 $\pm$ 1.0†
Systolic BP (mm Hg)*	105 $\pm$ 15	107 $\pm$ 8
Diastolic BP (mm Hg)*	64 $\pm$ 13	60 $\pm$ 17
Pulse rate (min ⁻¹)*	67 $\pm$ 6	70 $\pm$ 15

F, Female; M, male; BP, blood pressure.

*Mean $\pm$ SEM of 8 experiment day baselines.

†*P* < .05 versus healthy subjects.

RESULTS

Demographic and baseline physiologic data on the study population are shown in Table I. Mean baseline Q_{aw} values were significantly higher (*P* < .05), and mean percent predicted FEV₁ tended to be lower in asthmatic subjects. Within the healthy and asthmatic group, baseline mean Q_{aw} and FEV₁ were not different on the 8 visit days, and fluticasone and placebo pretreatment had no significant effect on these parameters (Tables II and III).

Mean systolic and diastolic blood pressure, pulse rate, and oxygen saturation were not influenced by any of the interventions.

Q_{aw}

In healthy subjects both the low and high doses of inhaled racemic and (R)-albuterol increased mean Q_{aw} values, and there was no difference between the high and low doses or racemic and (R)-albuterol, suggesting a maximum effect at the low doses of albuterol (Fig 1, A). The mean change in mean Q_{aw} values ranged between 14.6 $\pm$ 3.1 and 23.9 $\pm$ 3.7 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹. All mean postalbuterol Q_{aw} values were significantly different from the respective baselines (*P* < .05). The increases in Q_{aw} values were similar after placebo and fluticasone pretreatment.

In asthmatic subjects both doses of racemic albuterol and low-dose (R)-albuterol failed to change Q_{aw} values (mean Δ Q_{aw}, -4.4 $\pm$ 3.8, 2.0 $\pm$ 1.0, and -1.9 $\pm$ 3.9 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹; *P* = not significant) after placebo pretreatment (Fig 1, B). Only high-dose (R)-albuterol increased mean Q_{aw} values after placebo pretreatment (Δ Q_{aw}, +9.9 $\pm$ 4.0 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹; *P* < .05 vs low-dose albuterol). Pretreatment with fluticasone restored the airway vascular response to low-dose and high-dose racemic and low-dose (R)-albuterol (mean Δ Q_{aw}, +17.9 $\pm$ 3.8, +18.2 $\pm$ 4.1, and +22.5 $\pm$ 4.1 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹; *P* < .05) and potentiated the high-dose (R)-albuterol induced increase in Q_{aw} values (mean Δ Q_{aw} now +22.1 $\pm$ 4.1 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹ compared with +9.9 $\pm$ 4.0 μ L $\cdot$ min⁻¹ $\cdot$ mL⁻¹ after placebo; *P* < .05).

FEV₁

Mean FEV₁ did not significantly change after any treatment in healthy subjects (Fig 2, A). In asthmatic patients high-dose racemic albuterol and both (R)-albuterol doses increased mean FEV₁ after placebo pretreatment (Fig 2, B). Fluticasone pretreatment had no effect on albuterol-induced changes in FEV₁, except for low-dose racemic albuterol, where fluticasone potentiated the FEV₁ response.

TABLE II. Baseline mean Q_{aw} values ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$) before and after placebo or fluticasone pretreatment in healthy and asthmatic subjects (visits 1-8)

Treatment day	Healthy		Asthmatic	
	Before	After	Before	After
Racemic albuterol (0.6 mg)				
Placebo	31.6 $\pm$ 3.5	30.5 $\pm$ 3.5	44.6 $\pm$ 3.5	43.8 $\pm$ 3.8
Fluticasone	30.4 $\pm$ 3.9	29.3 $\pm$ 3.7	42.9 $\pm$ 3.9	38.9 $\pm$ 3.7
Racemic albuterol (1.25 mg)				
Placebo	31.5 $\pm$ 4.2	33.6 $\pm$ 4.2	39.9 $\pm$ 4.2	37.2 $\pm$ 4.2
Fluticasone	30.8 $\pm$ 3.4	33.1 $\pm$ 3.4	44.9 $\pm$ 3.4	47.5 $\pm$ 3.4
(R)-albuterol (0.3 mg)				
Placebo	32.7 $\pm$ 3.2	30.7 $\pm$ 3.8	44.1 $\pm$ 3.2	41.9 $\pm$ 3.8
Fluticasone	36.6 $\pm$ 4.4	31.7 $\pm$ 4.8	40.4 $\pm$ 4.4	43.9 $\pm$ 4.8
(R)-albuterol (0.6 mg)				
Placebo	36.3 $\pm$ 2.1	34.0 $\pm$ 3.6	38.0 $\pm$ 2.1	36.4 $\pm$ 3.6
Fluticasone	31.7 $\pm$ 3.6	33.7 $\pm$ 4.7	41.7 $\pm$ 3.6	40.9 $\pm$ 4.7

P = Not significant between respective before and after values (mean $\pm$ SE, n = 10) and P = not significant among pretreatment days in healthy and asthmatic subjects.

TABLE III. Baseline mean FEV₁ (L) before and after placebo or fluticasone pretreatment in healthy and asthmatic subjects (visits 1-8)

Treatment day	Healthy		Asthmatic	
	Before	After	Before	After
Racemic albuterol (0.6 mg)				
Placebo	2.87 $\pm$ 0.21	2.84 $\pm$ 0.12	3.23 $\pm$ 0.21	3.11 $\pm$ 0.13
Fluticasone	3.00 $\pm$ 0.16	3.00 $\pm$ 0.22	3.13 $\pm$ 0.12	3.14 $\pm$ 0.23
Racemic albuterol (1.25 mg)				
Placebo	2.80 $\pm$ 0.23	2.78 $\pm$ 0.41	3.18 $\pm$ 0.22	3.29 $\pm$ 0.43
Fluticasone	2.78 $\pm$ 0.21	2.81 $\pm$ 0.22	3.23 $\pm$ 0.22	3.18 $\pm$ 0.24
(R)-albuterol (0.3 mg)				
Placebo	2.79 $\pm$ 0.22	2.74 $\pm$ 0.13	3.09 $\pm$ 0.22	3.12 $\pm$ 0.14
Fluticasone	2.84 $\pm$ 0.25	2.85 $\pm$ 0.12	3.08 $\pm$ 0.23	3.10 $\pm$ 0.14
(R)-albuterol (0.6 mg)				
Placebo	2.77 $\pm$ 0.32	2.77 $\pm$ 0.22	3.15 $\pm$ 0.35	3.07 $\pm$ 0.21
Fluticasone	2.78 $\pm$ 0.22	2.81 $\pm$ 0.21	3.17 $\pm$ 0.21	3.07 $\pm$ 0.23

P = Not significant between respective before and after values (means $\pm$ SEs, n = 10).

DISCUSSION

These results confirm our previous observation that Q_{aw} values are increased in glucocorticoid-naïve patients with mild asthma.²¹⁻²³ The study also showed that albuterol responsiveness is blunted in asthmatic patients, which is again in keeping with similar previous findings.²² The increase and abnormal adrenergic responsiveness of Q_{aw} have been related to airway inflammation, supported by the finding that long-term treatment with an inhaled glucocorticoid or with an oral leukotriene modifier can reduce Q_{aw} values and that long-term glucocorticoid administration can restore albuterol responsiveness in these patients.^{3,9,24} These drug effects have been attributed to a reversal of inflammatory new vessel formation and changes in vasomotor tone and its regulation.^{25,26}

The present investigation has shown that a single low dose of an inhaled glucocorticoid can also restore Q_{aw} responsiveness to albuterol in patients with asthma. The glucocorticoid effect on albuterol occurred within 30 minutes, suggesting a nongenomic glucocorticoid action. In healthy subjects who have a significant vasodilator response to albuterol, the glucocorticoid-induced potentiation of adrenergic responsiveness was not observed, presumably because the maximum β_2 -adrenergic vasodilator effect was already reached after placebo pretreatment.

We have previously shown that the vasodilator effects of inhaled racemic and (R)-albuterol peak after 15 minutes and have

passed by 30 minutes and that the peak vascular effect of an inhaled glucocorticoid occurs between 30 and 60 minutes after inhalation and wanes by 90 minutes.^{9,17} The timing of the drug administrations in the present study was based on those observations. Although the vascular effect of adrenergic agonists is short lived, their effect on FEV₁ might last many hours. Therefore it was necessary to limit the experiment to one pretreatment/ β_2 -adrenergic agonist combination on a given visit. The respective visits were chronologically paired to minimize the possibility of changes in baseline parameters between the placebo and fluticasone visits for a given β_2 -adrenergic agonist in asthmatic subjects.

Although these experiments did not identify the mechanism whereby glucocorticoids acutely influence the vascular response to β_2 -adrenergic agonists, our observation is consistent with the previously reported nongenomic interaction between these agents *in vitro*. Glucocorticoids have been shown to inhibit the uptake of adrenergic agonists by airway vascular smooth muscle cells through the glucocorticoid's effect on glucocorticoid-sensitive organic cation transporters expressed in those cells.²⁷⁻³⁰

This glucocorticoid action, which is seen within minutes, theoretically could inhibit the disposal and increase the concentration of inhaled albuterol at β_2 -adrenergic receptor sites in airway tissue. As a result, the actions of inhaled adrenergic agonists should be acutely potentiated by glucocorticoids.^{31,32} This was seen in asthmatic patients in whom baseline Q_{aw} responsiveness

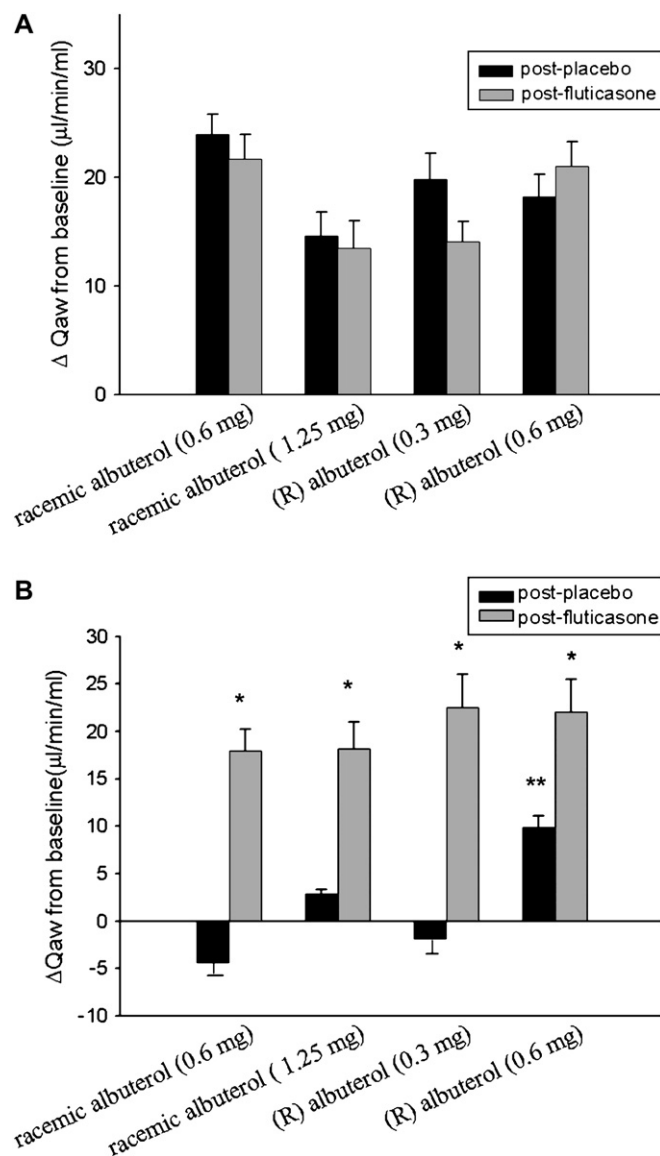


FIG 1. Airway blood flow reactivity to albuterol. The Q_{aw} response to racemic albuterol (0.6 mg and 1.25 mg) and (R)-albuterol (0.3 mg and 0.6 mg) with placebo or fluticasone pretreatment in healthy (A) and asthmatic (B) subjects is shown. Values are expressed as changes from the respective baseline (mean $\pm$ SE). * $P < .05$ versus corresponding placebo; ** $P < .05$ versus postplacebo albuterol, 0.6 mg.

to albuterol was blunted or attenuated. In healthy subjects the brisk Q_{aw} response after placebo pretreatment could not be potentiated by fluticasone pretreatment at the albuterol doses used in the study.

Presumably through the same mechanism, inhaled glucocorticoids acutely decrease Q_{aw} values in a dose-dependent fashion by inhibiting the disposal of locally released norepinephrine.^{9,17,33} At low glucocorticoid doses (eg, 100 μ g of fluticasone), vasoconstriction does not occur. This suggests that a decreased vascular clearance of the inhaled β_2 -adrenergic agonists was not responsible for the fluticasone-related potentiation of albuterol-induced vasodilation in the present study. We therefore believe that the observed potentiation of the vascular effects of the inhaled β_2 -adrenergic agonists by fluticasone was primarily due to its inhibitory action on local β_2 -adrenergic agonist disposal. This acute

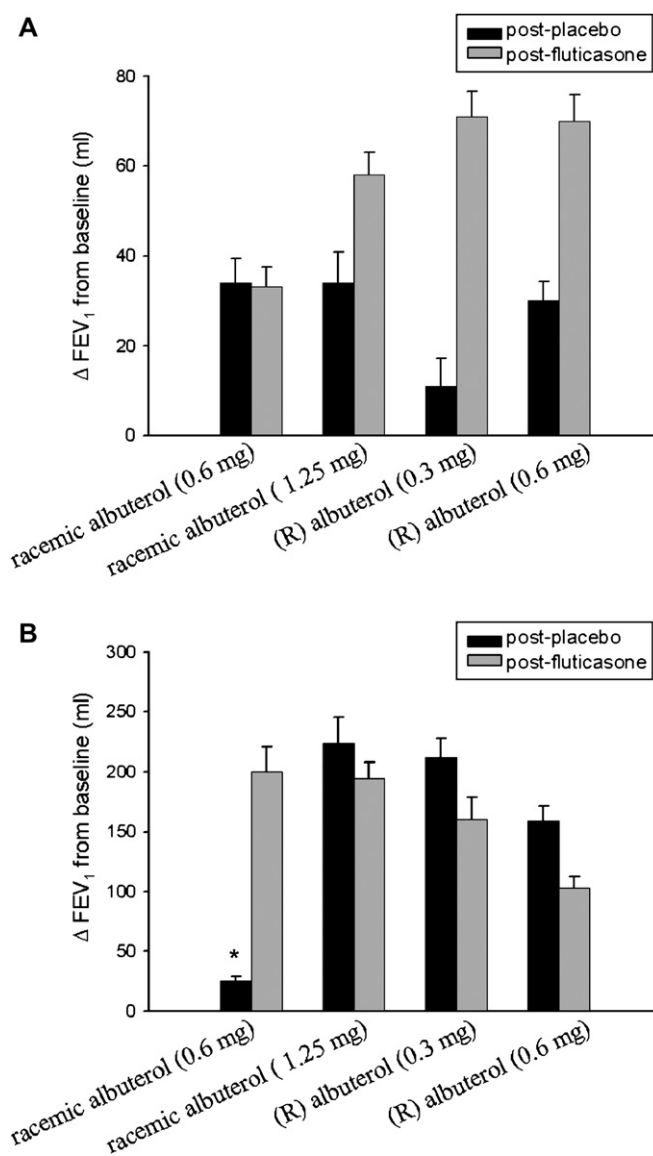


FIG 2. FEV₁ reactivity to albuterol. The FEV₁ response to racemic albuterol (0.6 mg and 1.25 mg) and (R)-albuterol (0.3 mg and 0.6 mg) with placebo or fluticasone pretreatment in healthy (A) and asthmatic (B) subjects is shown. Values are changes from the respective baseline expressed as milliliters (mean $\pm$ SE). * $P < .05$ versus corresponding placebo.

glucocorticoid-adrenergic interaction thus far has not been reported *in vivo*.

Our observation might be clinically relevant in that it raises the possibility of coadministering a glucocorticoid with an adrenergic agonist to potentiate the adrenergic agonist's immediate biologic effect. However, several questions must first be addressed before this could become a clinical reality.

First, it should be shown that coadministration of an adrenergic agonist with a glucocorticoid is as effective as glucocorticoid pretreatment, as tested in the present investigation. Second, it would be important to find out whether long-acting β_2 -adrenergic agonists are also substrates for the glucocorticoid-sensitive organic cation transporters and responsive to glucocorticoid potentiation. Third, patients with more severe airflow obstruction should be studied to determine whether airway smooth muscle is also sensitive to this glucocorticoid-adrenergic interaction

because in asthma airway smooth muscle responsiveness is a more important therapeutic target than airway vascular smooth muscle. In the present study we studied patients with mild asthma because we wanted to include only glucocorticoid-naïve subjects. Albuterol-induced bronchodilation and its potentiation by fluticasone were therefore difficult to assess. Only the response to low-dose racemic albuterol was improved by fluticasone pretreatment, whereas the better baseline response to the other albuterol formulations was insensitive to fluticasone. Subjects with more severe asthma will have to be studied in the future to determine whether the acute glucocorticoid effect on adrenergic smooth muscle relaxation also includes airway smooth muscle.

We compared the sensitivity of both racemic albuterol- and (R)-albuterol-induced vasodilation and bronchodilation to glucocorticoid pretreatment. This was done because differences have been reported in plasma albuterol concentrations after inhalation of racemic and (R)-albuterol.³⁴⁻³⁶ Because this difference might be related to differential local metabolism of the 2 drugs in airway tissue, we expected that fluticasone pretreatment would have different effects on (R)-albuterol- and racemic albuterol-induced vasodilation and bronchodilation. This was not the case in healthy subjects because both forms and doses of albuterol generally induced similar changes in Q_{aw} and FEV₁ with or without fluticasone pretreatment. In asthmatic subjects the potentiating action of fluticasone on Q_{aw} was also similar. However, high-dose (R)-albuterol increased Q_{aw} values after placebo pretreatment, whereas high-dose racemic albuterol had no effect. We have no explanation why this difference was only seen at the higher doses, but one might speculate that in contrast to racemic albuterol, (R)-albuterol can overcome the blunted β_2 -adrenergic vasodilator response of asthmatic subjects. It is not known whether this difference between (R)-albuterol and racemic albuterol would still be seen at higher drug dosages.

In summary, this study has, for the first time, demonstrated an immediate glucocorticoid/ β_2 -adrenergic interplay *in vivo* that could be explored clinically. The observation adds a new dimension to the interaction of 2 commonly used airway drugs for which previously only genomic interaction had been shown *in vivo*.

Clinical implications: Our findings support the notion that an inhaled glucocorticoid can potentiate the pharmacologic effect of an inhaled β_2 -agonist in asthma.

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