In vivo effect of albuterol on methacholine-contracted bronchi in conjunction with salmeterol and formoterol

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Background: It has been shown in vitro that prior treatment with salmeterol and formoterol antagonizes the relaxant effect of albuterol in carbachol-contracted human bronchi.
Objectives: The primary aim of this study was to evaluate whether there is a potential in vivo interaction between long- and short-acting β_2-agonists in the presence of increased airway tone induced by methacholine. In addition, a post hoc analysis was made to evaluate the effects of β_2-adrenoceptor polymorphisms.
Methods: Sixteen asthmatic subjects (mean age [±SD], 39 [13] years; FEV_1, 81% [17%] of predicted value), all taking inhaled corticosteroids and having methacholine PD_{20} values of less than 500 μg, were randomized in double-blind, double-dummy, cross-over fashion to receive single doses of inhaled placebo, inhaled formoterol 12 μg, or inhaled salmeterol 50 μg followed 12 hours later by a single dose of inhaled albuterol 400 μg (low dose) or 1600 μg (high dose). Methacholine challenges were performed on each of 6 separate occasions 1 hour after albuterol.
Results: There was a greater numerical difference in geometric mean PD_{20} values between low- and high-dose albuterol after placebo dosing (671 μg vs 1080 μg, a 1.61-fold difference; $P < .05$) compared with low- and high-dose albuterol after formoterol dosing (660 μg vs 799 μg, a 1.21-fold difference; $P = .4$), or after salmeterol dosing (568 μg vs 847 μg, a 1.49-fold difference; $P = .055$). PD_{20} values with high-dose albuterol in combination with formoterol or salmeterol were numerically lower than those found with high-dose albuterol in combination with placebo, but they were not significantly different. There was a significant difference between PD_{20} values with low-dose albuterol after dosing with formoterol (PD_{20} = 660 μg, a 1.6-fold difference; $P < .05$) or with salmeterol (PD_{20} = 568 μg, a 1.9-fold difference; $P < .05$) compared with PD_{20} with high-dose albuterol after placebo dosing (PD_{20} = 1080 μg). Post hoc polymorphism analysis for pooled pretreatment with formoterol and salmeterol (excluding placebo pretreatment) showed significantly ($P < .05$) lower PD_{20} values with homozygous glycine-16 compared with heterozygous glycine/arginine-16 and significantly ($P < .05$) lower PD_{20} values with homozygous glutamate-27 compared with either heterozygous glutamate/glutamine-27 or homozygous glutamine-27.
Conclusion: Compared with placebo, both salmeterol and formoterol caused a significant degree of antagonism of albuterol-induced bronchorelaxation in methacholine-contracted bronchi in vivo. This interaction could be caused by prolonged occupancy of airway β_2-adrenoceptors by long-acting β_2-agonists or by early tachyphylaxis 12 hours after a single-dose exposure. The degree of albuterol protection was also related to β_2-adrenoceptor polymorphism. (J Allergy Clin Immunol 1999;103:816-22.)

Key words: *Asthma, bronchoprotection, antagonism, airways, methacholine, albuterol, salmeterol, formoterol, polymorphism*

Regular treatment with inhaled long-acting β_2-agonists has been shown to improve long-term control of asthma[1-3] and is now recommended as a controller therapy in addition to inhaled corticosteroids.[4,5] Regular use of long-acting β_2-agonists leads to the development of downregulation and subsensitivity of airway β_2-adrenoceptors, which occurs despite concomitant inhaled corticosteroid therapy.[6-11] Improvements in symptom control and peak expiratory flow are maintained during treatment with long-acting β_2-agonists, although subsensitivity may make the airways more vulnerable to bronchoconstrictor stimuli. It is not clear, however, how long-acting β_2-agonists interact with short-acting β_2-agonists used as reliever therapy.

We have previously shown that chronic dosing with salmeterol 50 μg or formoterol 12 μg results in desensitization of the acute bronchoprotection afforded by a single high dose of albuterol.[12] This phenomenon could be caused by the effects of either downregulation or an interaction caused by receptor occupancy. It has been demonstrated in vitro that prior treatment with salmeterol or formoterol attenuated the maximal relaxant effect of albuterol in carbachol-contracted human bronchi.[13] This antagonism of albuterol response was presumed to be caused by prolonged receptor occupancy by long-acting β_2-agonists. Indeed, the antagonism occurred to a greater degree with salmeterol than formoterol, which may be explained by the weaker intrinsic agonist activity of salmeterol as compared with formoterol. The consequences of competitive antagonism will tend to become most evident in the presence of increased bronchomotor tone, particularly for a partial agonist such as salmeterol. In this study the primary objective was to evaluate in vivo whether the long-acting β_2-agonists salmeterol and for-

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TABLE I. Demographic data at recruitment

Patient no.	Age (y)	Sex	FEV_1 (L)	FEV_1 (% predicted)	Steroid and dose (μg/day)	PD_{20} baseline (μg)	PD_{20} after ALB (μg)	Genotype* (codons 16 and 27)
1	51	F	2.41	102	BDP 400	194	1247	Het and Het
2	38	F	1.84	67	BDP 200	35	227	Gly and Glu
3	51	F	12.3	93	BUD 800	252	6400	Het and Het
4	29	F	3.13	93	BDP 400	23	766	Gly and Glu
5	20	M	2.63	64	BUD 400	24	529	Gly and Glu
6	19	F	3.76	107	BUD 200	77	581	Het and Het
7	48	F	1.35	65	BDP 1000	35	205	Gly and Het
8	47	F	2.1	73	FP 2000	123	716	Gly and Glu
9	39	F	2.9	95	BUD 800	212	1472	Het and Het
10	38	M	3.44	83	BDP 500	299	2756	Gly and Gln
11	26	F	3.26	112	BDP 400	483	6400	Het and Gln
12	33	F	2.06	67	BDP 400	368	2257	Gly and Het
13	32	F	2.45	78	FP 500	87	1745	Gly and Glu
14	61	F	1.16	57	FP 2000	62	450	Het and Het
15	57	M	2.44	69	BUD 200	45	620	Gly and Het
16	34	F	2.04	74	BDP 1200	86	518	Het and Gln
	38.9		2.45	81.2	712	98†	995†	

All values are arithmetic means except PD_{20} values, which are geometric means.
ALB, Albuterol; *BDP,* beclomethasone dipropionate; *BUD,* budesonide; *FP,* fluticasone propionate.
*At codon 16: *Gly,* Homozygous Gly16-Gly16; *Het,* heterozygous Arg16-Gly16. At codon 27: *Glu,* homozygous Glu27-Glu27; *Het,* heterozygous Glu27-Gln27; *Gln,* homozygous Gln27-Gln27.
†Prealbuterol versus postalbuterol PD_{20} protection ratio = 10.2-fold.

Abbreviations used
Arg: Arginine
CI: Confidence interval
Gln: Glutamine
Glu: Glutamate
Gly: Glycine

moterol antagonize the response to the short-acting β_2-agonist albuterol in terms of its protective effect in the presence of increased bronchomotor tone induced by methacholine. In addition, we performed a post hoc analysis to evaluate the effects of β_2-adrenoceptor polymorphism.

METHODS

Subjects

Sixteen subjects (13 women and 3 men; mean [±SD] age, 39 [13] years) with mild-to-moderate asthma, all taking inhaled corticosteroids with a median dose of 450 μg/day (Table I), were recruited to take part in a randomized, double-blind, cross-over study. All patients were reactive to methacholine with PD_{20} values of less than 500 μg and exhibited at least a 2 doubling-dose improvement in PD_{20} at 20 minutes after inhalation of 400 μg of albuterol at initial screening (Table I). All had stable asthma, according to the American Thoracic Society criteria,[14] for at least 3 months before the study, and none had received a course of oral corticosteroids or antibiotics during this time. All were using inhaled β_2-agonists for symptomatic relief (<2 puffs/day), 2 were receiving oral theophylline, and 2 were receiving long acting β_2-agonist therapy with salmeterol. Baseline spirometry at recruitment showed a mean (±SD) FEV_1 value of 2.45 L (0.71 L) and 81% (17%) of predicted value and a mean FEF_{25-75} value of 1.98 L/sec (0.87 L/sec) and 52% (19%) of predicted value. All subjects gave written informed consent, and the study was approved by the Tayside Committee on Medical Research Ethics.

On completion of the study, we also performed a post hoc genotype analysis of our study patients. This revealed 9 patients who were homozygous for glycine (Gly)-16 and 7 patients who were heterozygous for Gly-16/arginine (Arg)-16 at codon 16; 5 patients were homozygous for glutamate (Glu)-27, 8 patients were heterozygous for Glu-27/glutamine (Gln)-27, and 3 patients were homozygous for Gln-27/Gln-27 at codon 27. All patients who were homozygous for Glu-27 were also homozygous for Gly-16, which is in keeping with known linkage disequilibrium.[15]

Study design

This study had a randomized, double-blind, double-dummy, placebo-controlled, cross-over design. For the screening visit, the subjects attended the laboratory in the morning, having withheld their long-acting β_2-agonist and theophylline therapy for at least 48 hours and short-acting β_2-agonist therapy for at least 8 hours. All laboratory visits were performed at the same time of day within a 1-hour window. A methacholine challenge test was then performed. Those subjects with a PD_{20} value of less than 500 μg were allowed to recover spontaneously and had a second methacholine challenge after inhalation of 400 μg of albuterol. There were 5 subjects who did not recover spontaneously within 60 minutes, and these subjects came back on another day for their second challenge with albuterol. All the subjects who had at least a 2 doubling-dose improvement in PD_{20} value were eligible to be entered into the study. From the start of the run-in period until the end of the study, all short- and long-acting β_2-agonists were withdrawn and replaced with the study medication. Inhaled ipratropium bromide (Atrovent Forte pressurized metered-dose inhaler, 40 μg per puff; Boehringer Ingelheim, Bracknell, UK) was substituted as reliever therapy, which was withheld for at least 12 hours before each study visit.

After the initial run-in period of at least 5 days, the subjects attended the laboratory 12 hours after taking 1 puff each of either

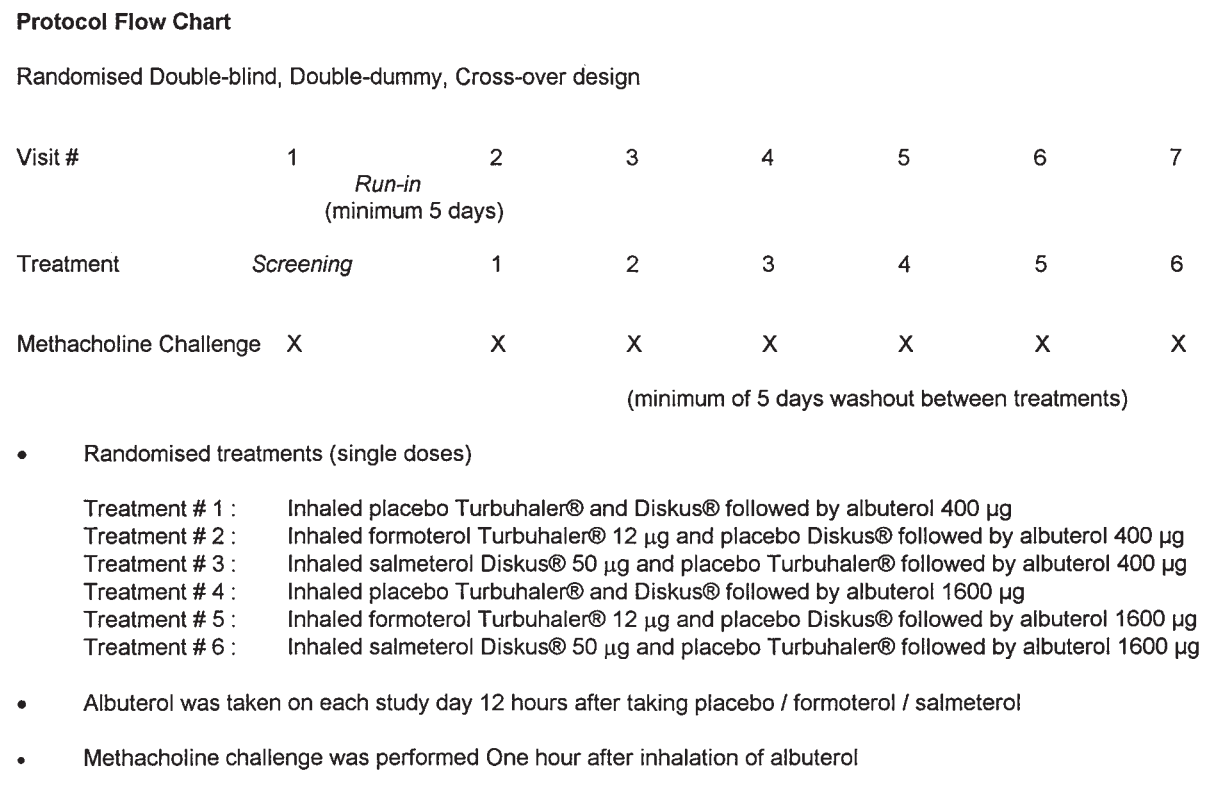

FIG 1. Flow chart of study protocol.

(1) inhaled formoterol 12 µg (eformoterol fumarate, 12 µg per puff, Oxis Turbuhaler; Astra Pharmaceuticals, King's Langley, UK) with 1 puff of a placebo Diskus, (2) inhaled salmeterol 50 µg (salmeterol xinafoate, 50 µg per puff, Serevent Diskus; Allen and Hanburys, Uxbridge, UK) with 1 puff of a placebo Turbuhaler, or (3) 1 puff of inhaled placebo (placebo Turbuhaler and placebo Diskus).

After measuring baseline spirometry at 12 hours after each randomized treatment, the subjects then inhaled either 400 µg or 1600 µg of albuterol (albuterol sulphate, 200 µg per puff, Ventolin Diskus; Allen and Hanburys) on 2 separate days by a second investigator who was not involved in the challenge testing. Spirometry was repeated 1 hour after inhalation and was followed immediately by a methacholine challenge. Thus the patients received the following randomized treatments on 6 separate occasions, each separated by a minimum wash-out period of at least 5 days: (1) placebo + albuterol 400 µg; (2) placebo + albuterol 1600 µg, (3) formoterol + albuterol 400 µg, (4) formoterol + albuterol 1600 µg, (5) salmeterol + albuterol 400 µg, and (6) salmeterol + albuterol 1600 µg (Fig 1).

Spirometry

Spirometry was performed according to American Thoracic Society criteria[16] by using a Vitalograph compact spirometer (Vitalograph Ltd, Buckingham, UK) with a pneumotachograph head and pressure transducer and on-line computer assisted determination of FEV_1 and FEF_{25-75}. The subjects performed 3 forced expiratory maneuvers from total lung capacity to residual volume, and the best test value was used in the analysis. The spirometer was calibrated daily with a Vitalograph 1-L precision syringe.

Methacholine bronchial challenge test

The methacholine bronchial challenge was performed by using a standardized computer-assisted dosimetric method as previously described.[17] In brief, methacholine was administered in doubling cumulative doses from 3.125 to 3200 µg given at 5-minute intervals until a 20% fall in FEV_1 was recorded. If FEV_1 did not show a 20% drop when a cumulative dose of 3200 µg had been inhaled, the PD_{20} was calculated by using a computer-assisted curve-fitting package (Biolab Assistant 1.1, University of Dundee, UK). If the curve fitting gave a PD_{20} value of greater than 6400 µg, a censored PD_{20} value of 6400 µg (double the maximum cumulative dose) was used for that test for the purpose of statistical analysis.[18]

Identification of β_2-adrenoceptor polymorphisms

β_2-Adrenoceptor polymorphisms at codons 16 and 27 were identified as previously described.[19] In brief, genomic DNA was extracted from whole blood and a 234-bp fragment generated by PCR, which spanned the regions of interest. Genotype was determined by allele-specific oligonucleotide hybridization with probes homologous for the Arg-16, Gly-16, Gln-27, or Glu-27 forms of the receptor.

Statistical analysis

The study was powered at the 80% level (β-error = 0.2) to detect a 2-fold difference (1 doubling dose) in PD_{20} between different treatments, with the α-error set at 0.05 (2-tailed). The data for PD_{20} were log-transformed to normalize their distribution before analysis. The change in PD_{20} was calculated as the geometric mean (expressed as micrograms) and as the geometric fold ratio (95% confidence interval [CI]) for protection compared with placebo and between low- and high-dose albuterol. The statistical analysis was performed by multifactorial analysis of variance followed by Bonferroni multiple-range testing with subject, treatment, period, and visit as factors. A probability *(P)* value of less than .05 (2-tailed)

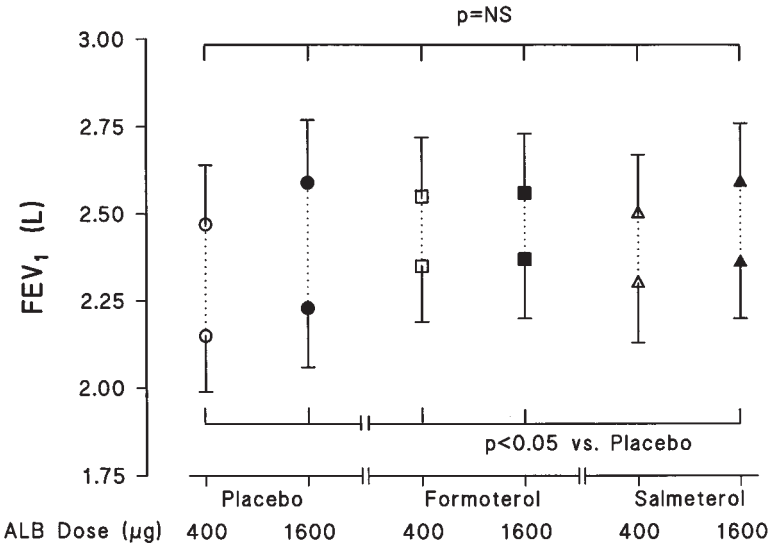

FIG 2. Mean (SE) FEV_1 values before (open symbols) and 1 hour after (filled symbols) inhalation of albuterol (ALB) and at 12 hours after a single dose of placebo, formoterol 12 μg, or salmeterol 50 μg. Prealbuterol and postalbuterol values are joined by a dotted line. There were no significant differences between postalbuterol values (ie, before challenge).

was considered significant. The analysis was performed with Statgraphics statistical software package (STSC Software Publishing Group, Rockville, Md). Analysis of the effects of β_2-adrenoceptor polymorphism was performed as a post hoc analysis by using Bonferroni multiple range testing.

RESULTS

Prechallenge spirometry

FEV_1 was significantly ($P < .05$) higher 12 hours after the inhalation of formoterol or salmeterol as compared with placebo, apart from one salmeterol treatment in which the numerical trend did not achieve statistical significance before inhalation of albuterol (Fig 2). There was a significant improvement in FEV_1 between prealbuterol and postalbuterol values after previous placebo dosing. However, the postalbuterol values (ie, prechallenge values) were not significantly different over all of the 6 visits (Fig 2).

Methacholine bronchial challenge

For within-treatment comparisons after placebo dosing, there was a greater increase in PD_{20} after albuterol 1600 μg compared with albuterol 400 μg (as geometric mean PD_{20}) (1080 μg vs 671 μg; $P = .05$), amounting to a 1.61-fold difference (95% CI, 1.13-fold to 2.9-fold), compared with high-dose versus low-dose albuterol after formoterol dosing (799 μg vs 660 μg, a 1.21-fold difference; $P = .4$) and after salmeterol dosing (847 μg vs 568 μg, a 1.41-fold difference; $P = .055$) (Fig 3).

For between-treatment comparisons, there was a significant ($P < .05$) difference between the PD_{20} values with low-dose albuterol after salmeterol or formoterol dosing in comparison with PD_{20} with high-dose albuterol after placebo dosing ([placebo + albuterol 1600 μg] = 1080 μg vs [formoterol + albuterol 400 μg] = 660 μg, a 1.64-fold difference; 95% CI, 1.08-fold vs 2.49-fold; and [placebo + albuterol 1600 μg] = 1080 μg vs [salmeterol + albuterol 400 μg] = 568 μg, a 1.9-fold difference; 95% CI, 1.25-fold to 2.9-fold). With high-dose albuterol, the PD_{20} value after formoterol or salmeterol dosing did not achieve the same level compared with placebo with high-dose albuterol, although this was not significant ([placebo + albuterol 1600 μg] PD_{20} = 1080 μg vs [formoterol + albuterol 1600 μg] = 799 μg; and [salmeterol + albuterol 1600 μg] PD_{20} = 847 μg) (Fig 3).

Genotype effects

When pretreatments with active medications (ie, excluding placebo pretreatment) were pooled together, the post hoc genotype analysis showed that at codon 16 the subjects who were homozygous for Gly/Gly had significantly ($P < .05$) lower PD_{20} values with both low- and high-dose albuterol compared with heterozygous Gly/Arg subjects (for albuterol 400 μg: Gly/Gly 506 μg vs Gly/Arg 780 μg, a 1.54-fold difference, 95% CI, 1.06-fold to 2.23-fold; for albuterol 1600 μg: Gly/Gly 608 μg vs Gly/Arg 1212 μg, a 2.0-fold difference, 95% CI, 1.41-fold to 2.80-fold) (Fig 4). Similarly, at codon 27 the subjects who were homozygous for Glu/Glu had significantly ($P < .05$) lower PD_{20} values with both low- and high-dose albuterol (for pooled active pretreatments) compared with those who were heterozygous for Glu/Gln and those who were homozygous for Gln/Gln (for albuterol 400 μg: Glu/Glu 366 μg vs Glu/Gln 743 μg, a 2.03-fold difference, 95% CI, 1.20-fold to 3.45-fold and Glu/Glu 366 μg vs Gln/Gln 859 μg, a 2.35-fold difference, 95% CI, 1.20-fold to 4.62-fold; and for albuterol 1600 μg: Glu/Glu 454 μg vs Glu/Gln 1074 μg, a 2.36-fold differ-

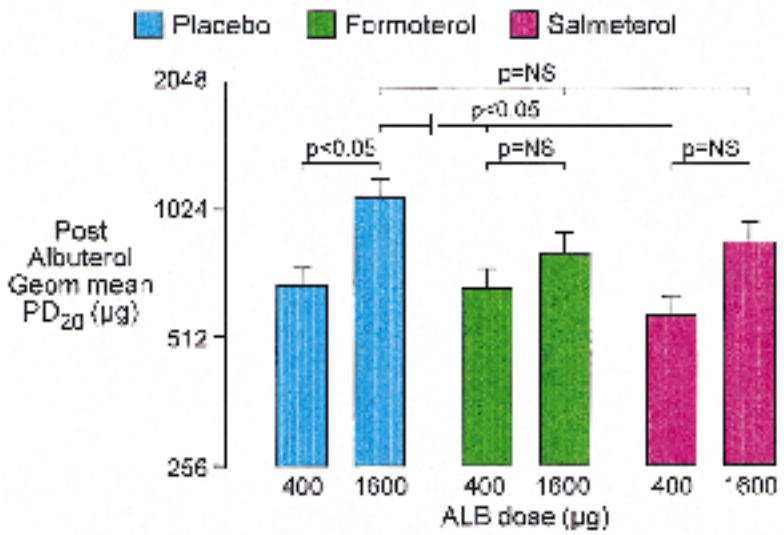

FIG 3. Methacholine PD_{20}. Geometric mean (SE) values for PD_{20} methacholine after low- and high-dose albuterol *(ALB)* treatment following dosing with placebo, formoterol, or salmeterol. Data for PD_{20} are plotted on a Log_2 scale to depict doubling doses of methacholine.

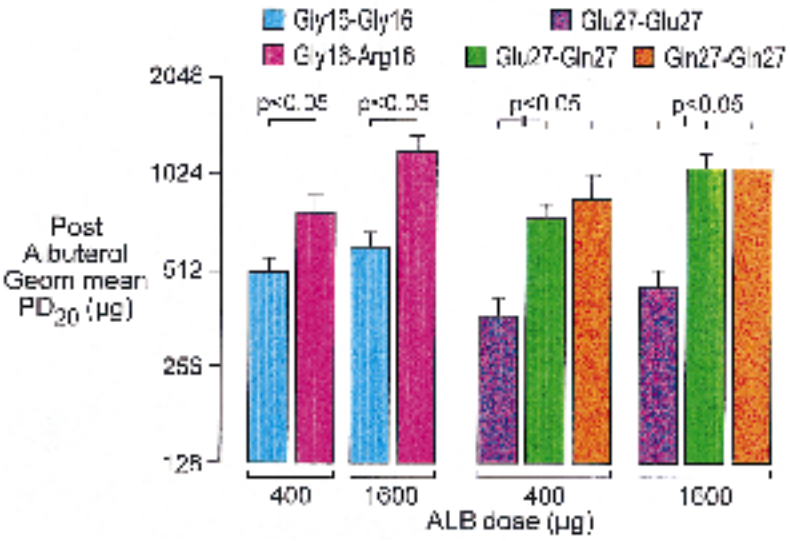

FIG 4. Effect of β_2-adrenoceptor polymorphisms for pooled active treatments (formoterol and salmeterol) characterized according to response to low- or high-dose albuterol *(ALB)*. Geometric mean (SE) methacholine PD_{20} values are depicted according to polymorphism at codons 16 and 27 of the β_2-adrenoceptor. The data are shown on a Log_2 scale to show doubling doses of methacholine.

ence, 95% CI, 1.45-fold to 3.85-fold and Glu/Glu 454 µg vs Gln/Gln 1088 µg, a 2.4-fold difference, 95% CI, 1.28-fold to 4.47-fold) (Fig 4).

DISCUSSION

Our results have demonstrated that prior treatment with a single dose of salmeterol and formoterol antagonized the protective effect of albuterol against methacholine-induced bronchoconstriction. This was evident by the greater degree of improvement in PD_{20} between low- and high-dose albuterol alone when given on a background of placebo compared with responses on a background of formoterol or salmeterol. Furthermore, the response to high-dose albuterol in conjunction with formoterol and salmeterol was numerically lower than the response to high-dose albuterol after placebo. The antagonism was seen despite both salmeterol and formoterol treatments producing significant bronchodilatation at 12 hours after dosing, and there was no attenuation of the subsequent bronchodilator response to albuterol. However, it should be pointed out that our patients had relatively mild asthma in terms of potential room for improvement in response to albuterol. Indeed,

the patients were screened for eligibility on the basis of their PD_{20} protected to albuterol and not their FEV_1 reversibility.

We accept the limitations of our study in that we did not determine the bronchoprotection produced by formoterol and salmeterol alone (ie, prealbuterol PD_{20}) for comparison with the addition of albuterol to these drugs. In a previous study Rabe et al[20] showed that 12 hours after a single dose, formoterol 12 μg and salmeterol 50 μg produced a 4.7-fold and 5.8-fold protection, respectively, against methacholine challenge compared with placebo. This compares to a 10.2-fold protection for prealbuterol versus postalbuterol (400 μg) PD_{20} values shown at the screening visit. Hence it might have been expected for the combination of albuterol with salmeterol or formoterol to exhibit additive effects to a greater degree than albuterol alone, which was clearly not the case. This in turn points to the antagonism of albuterol response by prior treatment with either salmeterol or formoterol. It is conceivable that the observed differences in PD_{20} between low- and high-dose albuterol in conjunction with formoterol or salmeterol may have been significant with a larger sample size given that our study was powered to detect a 1 doubling-dose (2-fold) difference. At the same time, we did not believe that examining the effect of a higher dose of albuterol, such as 3200 μg (16 puffs) or 6400 μg (32 puffs), would have any clinical relevance because it is unlikely that the patients would be self administering such a large number of puffs of reliever therapy from a dry powder inhaler in the event of acute bronchoconstriction. Nevertheless, we are unable to say whether 1600 μg achieved the top of the dose-response curve for protection against methacholine-induced bronchoconstriction.

We did not measure unprotected methacholine PD_{20} before albuterol inhalation because this may have confounded the PD_{20} value of the challenge performed 1 hour later after inhalation of albuterol. This is because repeated methacholine challenges within a short space of time may result in tachyphylaxis of methacholine effectiveness, which would therefore make it difficult to assess the true degree of albuterol protection. It was evident that the postalbuterol FEV_1 values (before challenge) were not significantly different over all 6 visits. Thus effects of airway geometry in the subsequent methacholine response are unlikely to explain differences in albuterol protection because all 6 visits started from the same degree of airway calibre.

What is the possible mechanism of this antagonism between long-acting β_2-agonists and albuterol? It is conceivable that persistent occupancy of the airway β_2-adrenoceptors by the long-acting β_2-agonists would be the most likely explanation. It has been shown that β_2-agonists with a low degree of intrinsic efficacy (eg, salmeterol) would act as an antagonist in situations in which there is receptor competition with a higher efficacy agonist (eg, albuterol). This effect would become most evident in the presence of increased bronchomotor tone as, for example, in a methacholine-contracted airway.[21] However, because formoterol is almost a full agonist and has a higher intrinsic efficacy than albuterol, we would have expected it to exhibit additive effects in conjunction with albuterol in comparison to albuterol alone. Nonetheless, our results are in keeping with findings in healthy volunteers showing antagonism of extrapulmonary β_2-responses to fenoterol and exercise in the presence of both formoterol and salmeterol.[22]

Another possibility is that early tachyphylaxis may have occurred after single-dose exposure to long-acting β_2-agonists. This is supported by a study in which there was significant loss of methacholine protection at 12 hours after a single dose of salmeterol.[23] Indeed, the occurrence of tachyphylaxis might conceivably explain why the antagonism of albuterol response appeared to be the same despite the differences in agonist activity with formoterol and salmeterol. It has been shown that regular treatment with salmeterol produces cross-tolerance to the bronchoprotective effects of albuterol.[11] Indeed, in our previous study desensitization of bronchoprotection against methacholine challenge induced by formoterol or salmeterol was not overcome by a 1600 μg dose of albuterol.[12]

The acute bronchodilator effect of albuterol was maintained at 12 hours after a single dose of both formoterol and salmeterol, and the FEV_1 value before the start of the methacholine challenge was similar among all treatments. It might have been expected that the bronchoprotective effect would also be similar among all treatments, but the bronchoprotective effect of albuterol was clearly better after placebo dosing compared with salmeterol and formoterol dosing. The likely explanation for this finding is that the bronchoprotective effect is more sensitive to changes in the β_2-adrenoceptor occupancy compared with bronchodilatory effect. This may also be the reason that bronchoprotective subsensitivity develops readily after regular treatment with long-acting β_2-agonists.[6,7,24]

The finding of a significant difference in the response between subjects according to their polymorphism of the β_2-adrenoceptor was a rather interesting observation of the study. It has been shown previously that subjects who are homozygous for Gly at codon 16 of the β_2-adrenoceptor have a propensity for the development of bronchodilatory subsensitivity after regular treatment with formoterol.[25] In our study the subjects who were homozygous for Gly at codon 16 also had significantly lower bronchoprotection against methacholine with both low- and high-dose albuterol therapy. Although the subjects who were homozygous for Glu at codon 27 showed a similar pattern, this may be explained by linkage disequilibrium with Gly-16 at Glu-27, with the former exhibiting the dominant effect on the phenotypic expression.[25] The effect of polymorphism on albuterol response would tend to suggest that this phenomenon was due to β_2-adrenoceptor downregulation, which may have occurred within 12 hours after single-dose exposure to salmeterol or formoterol.[23] Because the homozygous Gly-16 genotype has been reported to constitute up to 40% of the asthmatic population, these findings could

have important consequences in the treatment of acute asthma.[26,27]

In conclusion, we have shown that a single dose of formoterol and salmeterol produces antagonism of the protective effect of albuterol in the presence of methacholine-induced bronchoconstriction. This effect of albuterol was attenuated to a greater degree in subjects with the homozygous Gly genotype at codon 16 of the β_2-adrenoceptor. Further studies are indicated to assess whether this potential interaction between long- and short-acting β_2-agonists is clinically relevant in the setting of increased bronchomotor tone in acute severe asthma.

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