**β-Adrenergic Relaxation of Rabbit Tracheal Smooth Muscle: A Receptor Deficit That Improves with Corticosteroid Administration**

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ABSTRACT

β-Adrenergic agonists are potent relaxing agents of airway smooth muscle; however, they are often incapable of fully reversing agonist-mediated contractions. The present study was designed to quantitate the relationship between β-adrenergic receptor binding, signal transduction, and relaxation in rabbit tracheal smooth muscle (TSM). TSM segments contracted with acetylcholine to 25 to 75% maximal contraction were relaxed with cumulative administration of isoproterenol (ISO). A β-adrenergic receptor "deficit" was found, such that incomplete relaxation was achieved with full receptor occupancy. Binding studies with [3H]dihydroalprenol demonstrated a β-adrenoceptor density of 33.1 ± 8.6 fmol/mg protein in control TSM. Paired studies were performed in TSM from rabbits treated with dexamethasone. Relative to control tissues, dexamethasone-treated TSM displayed twice as much relaxation and cAMP production in response to ISO and twice the β-adrenoceptor density (82.2 ± 12.3 fmol/mg protein). Dexamethasone did not affect Gs function, as assessed by the degree of functional antagonism exerted by acetylcholine on ISO-induced relaxations, or β-adrenoceptor-Gs coupling, as reflected in high-affinity β-agonist binding. Collectively, these results demonstrate that corticosteroid administration exerts parallel potentiating effects on β-adrenoceptor expression and function in rabbit airway smooth muscle.

β-Adrenergic agonists are the principal bronchodilators used in the treatment of asthma. Nevertheless, we and others have shown that they are often incapable of fully relaxing contracted airway segments (Aberg et al., 1973; Brink et al., 1980; Hayashi and Toda, 1980; Lucchesi et al., 1990; Varlotta and Schramm, 1994; Schramm et al., 1995a). The β-adrenergic receptor belongs to a group of transmembrane-signaling proteins that are coupled to their effector enzymes by specific GTP-binding nucleotide proteins (G proteins). β-Adrenoceptor proteins are linked via a stimulatory G protein (Gs) to adenylyl cyclase, an enzyme whose activation results in the generation of the second messenger, cAMP. cAMP, in turn, activates a number of signaling pathways that result in smooth muscle relaxation. Certain calcium-mobilizing contractile agonists, particularly muscarinic cholinergic agonists (e.g., acetylcholine [Ach]), directly inhibit adenylyl cyclase activity through activation of intermediary G proteins (Torphy et al., 1983; Sankary et al., 1988). Increased activity of this muscarinic antagonistic pathway, resulting from increased M2 muscarinic receptor expression in airway smooth muscle (Emala et al., 1995), accounts for the impaired β-adrenergic responsiveness seen in the Basenji greyhound (Lindeman et al., 1991; Emala et al., 1995) and in the sensitized guinea pig model of asthma (Wills-Karp and Gilmour, 1993). Similarly, proinflammatory cytokines have been shown to attenuate β-agonist-mediated relaxation of rabbit tracheal smooth muscle (TSM) segments via enhanced expression of Gq proteins (Hakonarson et al., 1996).

By uncoupling the β-adrenoceptor from adenylyl cyclase, Gq-activating agonists decrease the number of functionally active β-adrenoceptors. For some receptor-signaling systems, a receptor reserve exists, such that an agonist’s maximum response can be achieved at a fraction of maximal receptor binding. The additional receptors are “spare” or “in reserve”, and their inactivation or loss does not affect the maximum agonist response (Ruffolo, 1982). Conversely, an inadequate receptor number may limit responsiveness. The inability of isoproterenol (ISO) to fully relax precontracted TSM suggests that there may be a lack of receptor reserve for β-adrenoceptors in airway smooth muscle. If β-agonist-mediated effects were β-adrenoceptor limited, then any potential increase in β-adrenoceptor density would enhance the β-agonist effect.

Methylprednisolone has been shown to restore β-adrenerg-
ergic responsiveness in Basenji greyhounds (Sauder et al., 1992), although the mechanisms of this corticosteroid effect have not been fully elucidated. Corticosteroid administration has been shown to increase β-adrenoceptor density in human leukocytes (Davies and Lefkowitz, 1980), rat and fetal rabbit lung homogenates (Mano et al., 1979; Cheng et al., 1980; Mak et al., 1995a), cultured human lung cells (Fraser and Venter, 1980; Nakane et al., 1990), and human peripheral lung tissue (Mak et al., 1995b). This effect is mediated through enhanced transcription of β-adrenoceptor genes (Mak et al., 1995a,b). These biologic influences of glucocorticoids on β-adrenoceptor expression are paralleled by the long-standing clinical impression that corticosteroids enhance the attenuated physiologic response to β-adrenergic stimulation in asthmatic individuals (Ellul-Micallef and French, 1975; Goldie et al., 1986). If airway smooth muscle is a β-adrenoceptor-limited system, then any increase in β-adrenoceptor expression elicited by corticosteroids should be directly reflected in enhanced cAMP generation and increased relaxation. The present study was designed to investigate the receptor reserve relationship for β-adrenergic stimulation in ACh-contracted TSM and to determine the effects of corticosteroids on β-adrenergic receptor expression, reserve, and function. Our findings demonstrate the presence of a β-adrenergic receptor “deficit” in muscarinically contracted rabbit TSM that is improved with corticosteroid administration.

Materials and Methods

Pharmacologic Receptor Occupancy Studies. TSM ring segments of 8 to 10 mm in length were isolated from adult New Zealand White rabbits sacrificed by systemic air embolism following anesthesia with xylazine (9 mg/kg) and ketamine hydrochloride (50 mg/kg). Corticosteroid-treated rabbits received daily i.m. injections of dexamethasone (5 mg/kg) for 2 days before sacrifice. The animal protocol was approved by the institutional Animal Care Committee, and all rabbits were cared for according to standards outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Airway segments were cleaned of loose connective tissue and were suspended between stainless steel triangular supports in siliconized Harvard 20-ml organ baths, such that the tube formed by the segment ran horizontally and the plane of the posterior trachealis muscle was aligned parallel to the supports. The lower support was secured to the base of the water bath; the upper support was attached to a Grass PT.03C force transducer from which isometric tension was continuously displayed on a multichannel recorder. The TSM segments were bathed in modified Krebs-Ringer solution [125 mM NaCl, 14 mM NaHCO3, 4 mM KCl, 2.25 mM CaCl2, (2H2O), 1.46 mM MgSO4 (7H2O), 1.2 mM NaH2PO4 (H2O), and 11 mM glucose] aerated with 5% CO2 balance O2, at pH 7.3 to 7.4, and a temperature of 37°C. A passive tension of 1.5 to 2.0 g was obtained for each tissue from sequential comparison of relaxations from low (i.e., 20–30% maximal), medium (~50%), and high (60–80%) levels of precontraction, and the tissue’s average Ks value was calculated. Third, β-adrenergic receptor reserve was characterized by the ratio of the above-mentioned Ka value to the EC50 concentration obtained from the initial relaxation of the half-maximal muscarinic contraction. Fourth, fractional receptor occupancy (B/Bmax) was determined for each administered ISO concentration according to the ratio [ISO] ([ISO] + Ka) and relaxation responses from half-maximal contractions were replotted as a function of fractional receptor occupancy.

Radioligand-Binding Studies. TSM segments from control and dexamethasone-treated animals (n = 3–4 for each assay) were stripped of epithelium and homogenized in iced 50 mM Tris-HCl buffer (pH 8.3 at 25°C) containing 2.5 mM MgCl2, 1 mM EDTA, and 1 mM dithiothreitol. Following an initial low-speed centrifugation (1,000g) to sediment large unsuspended fragments, the TSM homogenates were centrifuged for 12 min at 35,000g. The resultant pellets were resuspended in fresh buffer at a protein concentration of ~2 mg/ml. Aliquots containing ~200 μg of protein were prepared in triplicate and were exposed for 120 min at 4°C to five or six concentrations of [3H]dihydroalprenolol (DHA; 0.1–20 nM; 91 Ci/mmol). Bound ligand was isolated by rapid filtration over glass-fiber filters (Whatman GF/C; Tewksbury, MA) prewashed in 50 mM Tris-0.1% BSA buffer. The filters were rinsed three times with 4 ml Tris-BSA buffer under low vacuum to separate free and bound [3H]DHA, and the retained receptor-bound radioactivity was counted by liquid scintillation spectrophotometry. Nonspecific binding was measured in the presence of 10 μM propranolol. Maximal binding (Bmax) and binding affinity (Ka) were determined by iterative, nonlinear curve fitting (LIGAND) for one- and two-site models. In all cases, two-site models provided no better fits to the data than one-site binding. Bmax values were normalized to total protein content in the assays, as determined by Lowry’s method (Lowry et al., 1951) with BSA as the standard.

For each administered concentration of ISO, the values of fractional receptor occupancy calculated by receptor reserve analysis in control and steroid-treated TSM were multiplied by the respective values for β-adreceptor Bmax to determine absolute receptor occupancy (i.e., absolute occupancy in femtomoles per milligram protein = receptor reserve B/Bmax × radioligand Bmax in femtomoles per milligram protein). The relaxation response to each concentration of ISO was then replotted as a function of the total number of receptors occupied by that concentration. cAMP Assay. In separate studies, the levels of cAMP generated by varying concentrations of ISO (10−8 to 10−4 M) were determined in triplicate with a commercially available radioimmunoassay kit using [3H]cAMP as a tracer (Amersham International, Little Chalfont, UK). TSM segments from control and dexamethasone-treated rabbits (n = 5 each) were treated with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (10 μM) for 30 min before ISO administration. Thereafter, tissues were exposed to a single concentration of ISO for 1 min and then homogenized. Tissue cAMP levels were determined by displacement of [3H]cAMP from the commercial binding protein (Schramm et al., 1995b). The tissues’ protein-con
centrations were measured as described above, and cAMP measurements were expressed in units of picomoles per milligram tissue membrane protein.

**Analyses.** Results are expressed as means ± S.E. Statistical analyses were performed on means data by unpaired *t* tests. Dose-response curves were compared by repeated-measures ANOVA (StatView 4.5; Abacus Concepts, Berkeley, CA). Linear regressions were determined by the method of least-squares. The 95% CI for slopes and intercepts were calculated from respective mean and standard error values, and regressions were compared by *t* test analysis. *P* values of <.05 were considered statistically significant.

**Reagents.** Acetylcholine chloride, ISO hydrochloride, EDTA, di-thiothreitol, and 5′-guanylylimidodiphosphate were obtained from Sigma Chemical Co. (St. Louis, MO). Dexamethasone 21-(3,3-dimethylbutyrate) (Decadron) was obtained in sterile solution from the hospital pharmacy. [3H]DHA hydrochloride was obtained from Du-Pont-NEU (Wilmington, DE). Stock serial dilutions of ISO were prepared in 0.1 M ascorbic acid to prevent oxidative inactivation of the catecholamine. All drug concentrations are expressed as final solution concentrations.

**Results**

**Corticosteroid Effects on ISO-Induced Airway Relaxation and Receptor Reserve.** ISO-induced dose-dependent relaxation of rabbit TSM half-maximally precontracted with ACh. This relaxation was incomplete, however, such that maximal concentrations of ISO elicited only 30.2% relaxation of the muscarinic contractions (Table 1 and Fig. 1). Relative to control tissues, TSM from dexamethasone-treated rabbits demonstrated significantly enhanced relaxation to ISO (Fig. 1; *P* < .0001 by ANOVA). Airway sensitivity to ISO was not significantly increased in dexamethasone TSM, but the RT50max response was twice as great as in control tissues despite similar degrees of contraction of the two groups of tissue (Table 1). In contrast, dexamethasone treatment did not significantly alter the ACh dose-response relationship in rabbit TSM (*P* > .10 by ANOVA) or the maximal contractile response to ACh (Table 1).

Figure 2 illustrates the method of receptor reserve analysis for a representative control TSM segment. The percentage of relaxation responses were plotted for ISO at each of three levels of muscarinic contraction (Fig. 2A). As with the half-maximal contractions, the degrees of low and high contractions were similar in control (25.1 ± 2.9% and 68.6 ± 1.9% maximal tension, respectively) and dexamethasone TSM (20.2 ± 2.1% (*P* = .19) and 66.4 ± 2.7% (*P* = .51)). Concentrations of ISO eliciting equivalent percentage of relaxation responses were paired among the three dose-response relationships (i.e., ISOH versus ISO3M as in Fig. 2A, ISO3M versus ISO1, and ISO1 versus ISO5). Thereafter, double reciprocal plots were created between the paired, equipotent ISO concentrations (Fig. 2B). These plots were fit by linear regressions, such that the slope represented 1/q, where q equaled the remaining fraction of the original receptor population still active. As anticipated by receptor occupancy theory, the progressive receptor inactivation incurred by increasing muscarinic preconstriction was cumulative, such that qL > qM > qH. For the example given in Fig. 2B, only 25.7% of the β-adrenoceptors active at low levels of muscarinic contraction were still active at mid contraction (i.e., qL > qM > qH). Similarly, only 16.8% of the β-adrenoceptors active at mid levels of contraction were still active at high contraction (qM > qH). This 16.8% represents only 4.3% of the β-adrenoceptors active at the low levels of contraction (0.257 × 0.168 = 0.043 = qL > qH, which is very similar to the observed value of 6.3%). For each double reciprocal plot, the ratio of (slope − 1)/intercept yielded values for Kx, the apparent affinity constant for ISO. The Kx values were independent of the magnitude of the muscarinic contractions and were highly reproducible for each individual tissue. The average coefficient of variation (i.e., the standard deviation/mean value) for the three estimates of Kx from each tissue was 25.3%. The fraction of β-adrenoceptors occupied by any concentration of ISO (i.e., [ISO]) was defined by the ratio [ISO]/([ISO] + Kx). Accordingly, the relaxation responses to ISO could be plotted as a function of fractional receptor occupancy at each ISO dose (Fig. 2C).

Such analysis revealed the existence of a β-adrenergic “receptor deficit” in ACh-contracted TSM, such that full occupancy of receptors was associated with less-than-full relaxation of the muscarinic contraction. The average Kx value for ISO binding in control TSM was 1.09 ± 0.29 μM. This value was identical with the mean EC50 concentration of 1.22 ± 0.46 μM for ISO-induced relaxations of half-maximal muscarinic contractions. Kx/EC50 receptor occupancy ratios >1 indicate that half-maximal responses are achieved before half-
maximal binding and that there is a receptor reserve in the tissue. Muscarinically contracted rabbit TSM has no \(\beta\)-adrenergic receptor reserve (\(K_d/EC_{50}\) ratio = 0.9) and so demonstrates a direct relationship between the magnitudes of \(\beta\)-adrenoceptor binding and the relaxant response. Relative to control TSM, dexamethasone treatment resulted in no change in apparent ISO affinity (\(K_d\); Table 1). Because the \(EC_{50}\) value also was little affected, the average \(K_d/EC_{50}\) ratio was similar to that in control TSM. However, as shown in Fig. 3, dexamethasone TSM demonstrated a significantly enhanced relaxation response to fractional occupancy of the \(\beta\)-adrenoceptors with ISO. The relaxation-occupancy relationships could be approximated by straight lines for each set of tissues. The mean (and 95% CI) for the (% relaxation)/(fractional occupancy) slope in control TSM amounted to 29.2 (24.4–34.0). This slope was significantly increased in dexamethasone TSM, to 62.1 (52.6–71.6; \(P < .001\)).

**Corticosteroid Effects on \(\beta\)-Adrenergic Radioligand Binding.** \(^{[3H]}\)DHA was found to have specific single-site binding in TSM membrane homogenates from both control and dexamethasone-treated rabbits, as evidenced by LIGAND analysis, Hill coefficients near unity (0.929 ± 0.193 for control and 0.991 ± 0.110 for dexamethasone samples), and linear Scatchard graphs. Figure 4 depicts the cumulative Scatchard relationships of three studies, each with tissue from three or four control or steroid-treated rabbits. Relative to control TSM, dexamethasone treatment resulted in a slight decrease in \(^{[3H]}\)DHA binding affinity, with mean \(K_d\) values (95% CI) amounting to 2.86 nM (2.27–3.93 nM) in dexamethasone-treated and 1.56 nM (1.05–2.64 nM) in control TSM. Dexamethasone treatment also significantly enhanced total \(\beta\)-adrenoceptor density (i.e., maximal specific binding) in the TSM, with a mean (95% CI) \(B_{max}\) value of 82.2

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**Fig. 2.** Illustration of receptor reserve analysis in an individual control TSM segment. A, cumulative ISO relaxations were obtained on three separate occasions from the TSM contracted to 21% maximal ACh-induced tone (0.1 \(\mu\)M ACh; ●; ISO\(^L\)), 53% maximal tone (1.0 \(\mu\)M ACh; ■; ISO\(^M\)), and 77% maximal tone (3.0 \(\mu\)M ACh; ▲; ISO\(^H\)). ISO concentrations associated with equivalent relaxant responses were interpolated between each pair of contractile responses (as shown for ISO\(^L\) and ISO\(^M\) in the figure). B, double reciprocal plots are shown for equipotent 1/ISO\(^L\) versus 1/ISO\(^M\) (○) and 1/ISO\(^M\) versus 1/ISO\(^H\) (■) comparisons. The 1/ISO\(^L\) versus 1/ISO\(^M\) comparison is not shown because it is off scale. Its regression was \(y = 13.926 + 15.857x (r = 1.00)\), yielding values of 0.063 for q and 1.07 \(\mu\)M for \(K_d\). Note that the \(K_d\) values are similar for the three sets of analyses (coefficient of variation 38%) and that functional \(\beta\)-adrenergic uncoupling was cumulative, such that \(q_L > q_M > q_H (0.063 - q_H < q_L - q_M = (0.257) * q_M - q_H (0.168)\). C, fractional receptor occupancy was calculated for each ISO concentration [ISO] by the relationship [ISO]/([ISO] + \(K_d\)). The relaxant responses from half-maximal muscarinic contraction are replotted as a function of \(\beta\)-adrenoceptor occupancy.

**Fig. 3.** Comparison of relaxation-fractional receptor occupancy relationships to ISO in TSM segments half-maximally contracted with ACh. Relative to control TSM (○; dashed line; \(n = 11\)), dexamethasone-treated TSM (■; solid line; \(n = 12\)) showed enhanced relaxant responses beyond 40% receptor occupancy. Note that the magnitudes of relaxation are the same as those depicted in Fig. 1. Data represent means ± S.E.

**Fig. 4.** Comparison of Scatchard relationships for specific \(^{[3H]}\)DHA binding in TSM membrane preparations from control (○; dashed line) and dexamethasone-treated (■; solid line) rabbits. Note that dexamethasone administration resulted in a significantly greater \(B_{max}\) (i.e., x-intercept) and a slightly attenuated \(K_d\) value (i.e., −1/slope). Data represent combined results from three studies, each containing three or four control or steroid-treated rabbits and normalized for the amount of protein in each.
(60.4–113.5) fmol/mg protein, versus 33.1 (19.6–57.2) fmol/mg protein in control TSM (P < .005).

**Corticosteroid Effect on β-Adrenergic Agonist-Receptor Response Relationships.** Knowledge of the absolute numbers of β-adrenoceptors in the TSM allowed total receptor occupancy to be determined for each concentration of ISO and its corresponding fractional receptor occupancy. The percentage of relaxation responses were then plotted as a function of absolute receptor occupancy (Fig. 5). In contrast to the fractional receptor occupancy dose-response relationship (Fig. 3), dexamethasone treatment did not change the relaxant response to an absolute number of β-adrenoceptors activated by a given dose of ISO in rabbit TSM. The mean (95% CI) slope of the dexamethasone response, −0.537 (−0.491 to −0.583) % relaxation/fmol/mg protein, was similar to the slope of −0.611 (−0.560 to −0.670) in control TSM (.10 < P < .05).

**Corticosteroid Effects on Functional Gs and Gi Activities.** The above-mentioned studies suggested that dexamethasone enhanced ISO-induced relaxation primarily through increasing the density of TSM β-adrenoceptor proteins. To determine whether the corticosteroid also affected the activities of Gs and Gi proteins on the β-adrenergic response, separate studies addressed the functional activities of these G proteins in the rabbit TSM. Gi activity was quantified by the degree of muscarinic antagonism of ISO-mediated relaxation. As shown in Fig. 6, the sensitivity to ISO (i.e., −log EC50) was inversely proportional to the magnitude of muscarinic contraction. Dexamethasone treatment resulted in a parallel upward shift in this functional antagonism relationship (t = 6.311 for comparison of elevations; P < .001), as would be expected by increasing the number of β-adrenoceptors. The regression slope was not changed in the steroid-treated TSM (−0.029 −log M/%Tmax relative to control TSM (−0.026 −log M/%Tmax). Thus, corticosteroid administration did not affect the modulatory influence of Gi proteins in determining the relaxant response to β-adrenoceptor stimulation in TSM.

**Fig. 5.** Comparison of relaxation-receptor occupancy relationships to ISO in TSM segments half-maximally contracted with ACh. Relative to TSM from control rabbits (○; dashed line; n = 11), TSM from dexamethasone-treated animals (●; solid line; n = 12) had similar relaxant responses to activation of an absolute number of receptors. The potentiated maximal response in the steroid-treated TSM was due to the increased number of β-adrenoceptors expressed following dexamethasone administration. Data represent means ± S.E.

**Fig. 6.** Comparison of muscarinic functional antagonism of ISO-mediated TSM relaxation. Relative to TSM from control rabbits (○; dashed line), TSM from dexamethasone-treated animals (●; solid line) demonstrated enhanced ISO sensitivity for any magnitude of contraction (upward displacement of the regression) but a similar degree of functional antagonism (parallel slope of the regression). Data represent −logEC50 values obtained from individual dose-response relationships at the corresponding degree of ACh-induced contraction (expressed as a percentage of the maximal contractile response).

The Gs influence was assessed by determining the ratio of high- to low-affinity ISO-binding sites in TSM. ISO displaced [3H]DHA binding in a dose-dependent fashion, characterized by two sites of high and low affinity (Fig. 7 and Table 2). Addition of the nonhydrolyzable GTP analog 5′-guanylylimidodiphosphate resulted in loss of the high-affinity binding sites and a shift of the inhibition curve to the right. TSM obtained from dexamethasone-treated rabbits had more high-affinity binding sites than control tissues but similar levels of low-affinity binding sites (Table 2). High- and low-affinity binding constants were not affected by corticosteroid treatment. Dexamethasone administration increased total β-adrenoceptor-binding sites 1.7-fold (P < .05), similar to what was observed in the [3H]DHA saturation-binding experiments. Although the fraction of high-affinity binding sites tended to be greater in dexamethasone TSM (59 ± 11%
of total β-adrenoceptors) than in control TSM (44 ± 10%), this difference was not statistically significant (P > .2). In an attempt to compare radioligand binding data to the receptor occupancy studies, crude apparent $K_A$ values ($K_A'$) for ISO were derived for control and dexamethasone TSM by the formula \[ [K_A'] = (K_{A, \text{HIGH}} + B_{\text{HIGH}}) + (K_{A, \text{LOW}} + B_{\text{LOW}})/(B_{\text{HIGH}} + B_{\text{LOW}}) \]. Of interest, these apparent $K_A'$ values amounted to 0.50 μM in control and 0.96 μM in dexamethasone-treated TSM, in close agreement with the $K_A$ values obtained by receptor reserve analysis.

Corticosteroid Effect on β-Adrenergic cAMP Generation and cAMP-Relaxation Relationships. The increased relaxant response in dexamethasone TSM was mirrored in studies of cAMP generation in response to ISO. Baseline cAMP levels were similar in TSM homogenates from unstimulated control (15.9 ± 2.8 pmol/mg protein) and dexamethasone-treated rabbits (14.8 ± 1.4; P = .77; n = 5). In contrast, the cAMP response to ISO was significantly potentiated in dexamethasone TSM (P = .001; Fig. 8). The maximum amount of generated cAMP was 1.7-fold higher in dexamethasone TSM (P = .008), but cAMP sensitivity to ISO was unchanged. As shown in Fig. 9, a direct relationship was found between the relaxation elicited by a given amount of ISO and the cAMP generated by that concentration. The tissue’s relaxant responsiveness to cAMP could be characterized, therefore, by the slope of this relationship. These slopes were similar in control and dexamethasone-treated TSM, amounting to 2.17% relaxation/(pmol cAMP/mg protein) (95% CI; 1.20–3.14) and 1.60 (0.66–2.54), respectively. Thus, dexamethasone treatment increased both ISO-mediated TSM relaxation and cAMP generation, without affecting the cAMP-relaxation response relationship in rabbit TSM.

Discussion

There is a limitation to β-adrenergic signal transduction in airway smooth muscle, such that incomplete relaxation plateaus occur at ISO concentrations of 1 to 100 μM in muscarinically contracted rat (Frossard and Landry, 1985) and canine (Sankary et al., 1988) TSM and in either muscarinic- or KCl-contracted rabbit TSM (Varlotta and Schramm, 1994; Schramm et al., 1995a). The site of this limitation could be downstream from β-adrenoceptor-adenyl cyclase signaling, or it could be related to a relative deficiency in β-adrenoceptor density in TSM. The present study demonstrates that there is a β-adrenergic “receptor deficit” in rabbit TSM, such that full receptor occupancy is associated with only partial relaxation. This deficit is magnified by the effective uncoupling of β-adrenoceptors from adenyl cyclase, as a result of $G_\alpha$ activation by muscarinic agonists.

Receptor reserve theory depends on comparison of agonist responses before and after irreversible inhibition of a fraction of the agonist’s receptors, classically achieved with agents that either covalently bind to the receptor or permanently alter it (Furchgott and Bursztyn, 1967). Nevertheless, Buckner and Saini (1975) demonstrated that muscarinic functional antagonism could be used to derive apparent affinity constants for β-adrenergic agonists in guinea pig trachea. Our analysis supports this approach (Fig. 2). Linear double reciprocal plots were obtained for all of the three analyses in each tissue (i.e., between relaxations from low and middle, middle and high, and low and high acetylcholine-induced contractions), and the three resulting $K_A$ values had low coefficients of variation. Moreover, progressively greater contractions were associated with cumulative functional loss of

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Fig. 8. Comparison of cAMP dose-response relationships to ISO in TSM segments from control animals (■) and TSM from dexamethasone-treated rabbits (□; n = 5 in each group). Relative to control responses, ISO induced significantly more generation of cAMP in dexamethasone-treated TSM. Data represent means ± S.E. *, designates P < .05 versus paired matched control response.

Fig. 9. Comparison of relaxation responses to cAMP generation in TSM from control and dexamethasone-treated rabbits. The relaxation responses to given doses of ISO (Fig. 1) were paired to cAMP responses (Fig. 8) from the same doses in separate studies. Dexamethasone-treated TSM (□) demonstrated increased relaxation and cAMP production in response to ISO than control TSM (■). Nevertheless, the amount of relaxation achieved per picomole of cAMP generated was similar in dexamethasone TSM (solid line) and in control TSM (dashed line). Data represent means ± S.E.


β-adrenoceptors, as would be seen with progressive treatment with an irreversible antagonist. The $K_a$ value obtained for ISO in rabbit TSM (1.09 μM) was considerably greater than the $K_a$ value of 0.03 μM obtained with this methodology in guinea pig TSM (Buckner and Saini, 1975). However, it should be noted that guinea pig TSM is significantly more sensitive to ISO than rabbit TSM, with $EC_{50}$ concentrations of ~0.01 μM reported in carbachol-contracted TSM (Aberg et al., 1973). Thus, the $K_a/EC_{50}$ ratio is also close to unity in guinea pig TSM, suggesting the absence of a receptor reserve for β-adrenoceptors in guinea pig as well as rabbit airway smooth muscle. Similar findings have been reported for 5-hydroxytryptamine and histamine receptors in canine TSM, wherein maximal responses require activation of 78 and 88% of the respective receptors (Gunst et al., 1987). In contrast, maximal responses to ACh are obtained with activation of only 4% of receptors, indicating the presence of a very large muscarinic receptor reserve in TSM (Gunst et al., 1987).

Pretreatment of rabbits for 48 h with dexamethasone significantly increased the relaxant response to ISO in isolated TSM segments, with a doubling of maximal relaxation (Fig. 1). It is unlikely that this potentiating effect is related to glucocorticoid inhibition of tissue uptake and degradation of ISO (Varlotta and Schramm, 1994). Dexamethasone's half-life is 3.61 h in the rabbit (Ogiso et al., 1985), and so 99% of the administered dexamethasone should have been metabolized in the 24 h between the last dose and the animal's sacrifice. Even in the dexamethasone-treated TSM, however, ISO was unable to completely relax ACh-induced contractions. Dexamethasone treatment did not affect the apparent affinity of TSM for ISO, nor did it substantially change the $K_a/EC_{50}$ receptor reserve ratio. Nevertheless, when fractional receptor binding was calculated for each ISO concentration and the corresponding relaxant responses were plotted against fractional β-adrenoceptor occupancy, the slope of the relationship was 2.1-fold greater in TSM from dexamethasone-treated than control rabbits (Fig. 3). To account for this finding by receptor occupancy theory, dexamethasone treatment must have increased either the number of β-adrenergic receptors or the efficacy relationship between binding and relaxation (or a combination of both).

Saturation radioligand-binding studies demonstrated that in vivo treatment with dexamethasone doubled the number of β-adrenoceptors present in rabbit TSM (Fig. 4). This response is similar to the 1.7-fold increase in rat lung β-adrenoceptor density following 8 days of in vivo dexamethasone (Mak et al., 1995a) and the 1.6-fold increase after 17 to 24 h in peripheral human lung tissue (Mak et al., 1995b). The β-adrenoceptor density of 33.1 fmol/mg protein in control rabbit TSM was somewhat less than the level of 95.6 fmol/mg protein in canine TSM (Barnes et al., 1983), possibly related to species differences or to differences in preparation of the membrane samples. [3H]DHA bound to a single site in both control and dexamethasone TSM, with affinities similar to the $K_a$ values of 1.0 nM in canine TSM (Barnes et al., 1983) and 1.2 nM in human lung membranes (Lopes et al., 1991).

Knowledge of the absolute numbers of β-adrenoceptors in the TSM allowed total receptor occupancy to be determined for each concentration of ISO and its corresponding fractional receptor occupancy. When the relaxation-total occupancy relationships were compared, it was seen that the activation of a given absolute number of β-adrenoceptors by ISO resulted in the same relaxant response in control and dexamethasone-treated tissues (Fig. 5). The doubling in maximal relaxation from steroid treatment was related to the 2-fold increase in β-adrenoceptor density in TSM from dexamethasone-treated rabbits. There was no evidence of enhanced receptor-effector coupling.

These conclusions were supported by two additional lines of evidence. First, dexamethasone exposure did not influence the affects of $G_i$ or $G_s$ on ISO-induced relaxations. Increasing muscarinic stimulation resulted in similar degrees of $G_i$-mediated inhibition of ISO relaxation in control and dexamethasone-treated TSM (i.e., parallel regression lines in Fig. 6). The slopes of these regression lines are similar to what we have previously observed in rabbit TSM (Schramm et al., 1995a) and what has been reported in canine TSM (Torphy et al., 1983). Dexamethasone-treated TSM depicted a 1.5-fold increase in ISO sensitivity with half-maximal muscarinic contractions (Fig. 1). The parallel upward displacement of the functional antagonism regression line in Fig. 6 represents a similar increase in ISO sensitivity at all levels of muscarinic contraction in steroid-treated TSM, due to increased β-adrenoceptor density in the tissue. The number of high-affinity β-adrenoceptors was increased by dexamethasone treatment, but in proportion to the increase in total β-adrenoceptor expression. Because the proportion of high-affinity to total binding was not affected, dexamethasone treatment did not appear to alter the coupling relationship between β-adrenoceptors and $G_s$ proteins in rabbit TSM.

In addition, dexamethasone treatment did not affect the TSM relaxant response relationship to cAMP generation from β-adrenoceptor stimulation (Fig. 9). Any significant potentiation of cAMP-independent relaxant mechanisms (e.g., K+ channel activity) or downstream cAMP-dependent mechanisms (e.g., protein kinase A activity, intracellular Ca2+ fluxes, or Na+-K+ ATPase activity) would alter the slope of this relationship because more relaxation would occur for the amount of cAMP produced. In addition, if dexamethasone treatment had inhibited TSM phosphodiesterase activity, the corticosteroid's potentiating effect would have been attenuated in the cAMP assay (with 3-isobutyl-1-methylxanthine in both control and dexamethasone samples) and the % relaxation/cAMP slope would have been affected. Thus, although the present study cannot rule out any potential corticosteroid effects on downstream effectors of β-adrenergic relaxation, the demonstration that cAMP relaxation-response relationships are unchanged by dexamethasone treatment suggests that the potentiating action of corticosteroids on β-agonist-mediated airway relation is primarily localized to the β-adrenoceptor signaling level.

Topographical differences exist in the number of receptors and in functional responses in the airways. Rabbit airways, like those in the guinea pig (Wasserman and Mukherjee, 1988), demonstrate greater ISO relaxations in tracheal than bronchial segments (unpublished observations). Tracheal tissues from guinea pigs are also more sensitive than bronchial segments to ISO, despite increased β-adrenoceptor density in bronchial tissues (Duncan et al., 1982). Thus, β-adrenergic signal transduction (i.e., receptor-effector coupling) appears to be more effective in tracheal than bronchial smooth muscle. The potential mechanisms underlying this observation have not been investigated. In addition, although muscarinic contractions were used in this study as a tool to uncouple
beta-adrenoceptors from their signaling pathway for the receptor occupancy studies, we have previously observed that ISO is also incapable of fully relaxing rabbit TSM precontracted with KCl (Varlotta and Schramm, 1994; Schramm et al., 1995a). That ISO’s maximal relaxations of KCl-contracted TSM are similar in magnitude to those following half-maximal muscarinic contractions suggests that the beta-adrenoceptor deficit is not limited to the condition of muscarinic contractile stimulation. Moreover, in light of the enhanced G1 expression and activity in sensitized airway smooth muscle (Wills-Karp and Gilmour, 1993; Hakonarson et al., 1996), the existing beta-adrenoceptor deficit is likely to be exacerbated in asthmatic Airways.

In summary, the present study identified the presence of a receptor deficit for beta-adrenoceptors in muscarinically contracted rabbit TSM, such that full receptor activation elicited only partial relaxation of the contraction. Forty-eight hours of in vivo dexamethasone administration potentiated in vitro beta-adrenergic responsiveness in rabbit TSM segments. The TSM relaxant response to fractional beta-adrenoceptor occupancy was enhanced by dexamethasone treatment because of increased beta-adrenoceptor density in the airway smooth muscle. Dexamethasone administration did not affect either the relaxant response to absolute beta-adrenoceptor occupancy in rabbit tracheal smooth muscle or the relaxant response to beta-agonist-mediated cAMP generation. The enhancing effects of dexamethasone on beta-adrenergic relaxation of airway smooth muscle correlated with increased beta-adrenoceptor expression in the tissue. These findings support and provide a mechanism for the long-standing clinical impression that corticosteroids can enhance the airway relaxant response to beta-adrenergic stimulation.

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