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Methylprednisolone Prevents Propranolol-induced Airway Hyperreactivity in the Basenji-greyhound Dog

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To determine if corticosteroids would prevent β-adrenergic-antagonist-induced increases in airway reactivity, we evaluated the ability of chronic methylprednisolone administration to prevent propranolol-induced airway hyperreactivity to methacholine aerosol in the basenji-greyhound (BG) dog model of asthma. Initial studies included the measurement of lung resistance (R_L) and dynamic compliance (C_{dyn}) with and without propranolol pretreatment in 5 BG and 5 mongrel dogs. A single dose of propranolol (2 mg/kg) did not significantly alter airway reactivity in the mongrels. The dose of methacholine needed to increase R_L by 200% ($ED_{200}R_L$) was 0.20 ± 0.05 mg/ml (mean ± standard error of the mean [SEM]) in untreated and 0.18 ± 0.04 mg/ml in propranolol-treated mongrels. In contrast, propranolol significantly increased methacholine-reactivity in the BGs. The $ED_{200}R_L$ for methacholine was 0.17 ± 0.03 mg/ml in untreated and 0.05 ± 0.02 mg/ml ($P < 0.05$) in propranolol-treated BG dogs. Following the initial studies, the 5 BG dogs were given methylprednisolone (2 mg · kg^{-1} · day^{-1}) for 4 weeks, after which time propranolol no longer increased methacholine reactivity in the BGs. The $ED_{200}R_L$ was 0.16 ± 0.03 mg/ml after 4 weeks of methylprednisolone and 0.22 ± 0.06 mg/ml after propranolol administration in the BGs given 4 weeks of methylprednisolone treatment. The attenuation of propranolol-induced bronchoconstriction by corticosteroids may be a clinically useful intervention in asthmatic patients receiving β-adrenergic antagonists in the perioperative period. However, further studies are needed to define the effective dose and duration of corticosteroid therapy that is needed. (Key words: Allergy, asthma: airway reactivity; bronchoconstriction. Anesthetics, intravenous: fentanyl; thiopental. Antagonists, β-adrenergic: propranolol. Lung: dynamic compliance; pulmonary resistance. Pharmacology: methylprednisolone. Sympathetic nervous system, β-adrenergic receptor antagonist: propranolol.)

ISCHEMIC HEART DISEASE, cardiac dysryhthmias, hypertension, asthma, and chronic obstructive pulmonary disease (COPD) with a component of reversible airway obstruction are common conditions occurring in various combinations in patients requiring anesthesia. β-adrenergic antagonists are frequently used to treat the cardiovascular symptoms even though β-adrenergic antagonists increase pulmonary reactivity in patients with coexisting asthma and COPD.[1–3] When presenting for anesthesia, such patients often are receiving chronic treatment with β-adrenergic antagonists, the removal of which would produce adverse effects on the cardiovascular system.[4]

Glucocorticoids are known to potentiate β-adrenergic function[5] and are beneficial drugs in the treatment of asthma.[6] Therefore, glucocorticoids may reverse the increase in airway reactivity produced by β-adrenergic antagonists in asthma. First, we compared the effects of the β-adrenergic antagonist, propranolol, on airway reactivity to methacholine in control dogs and in the basenji-greyhound (BG) dog model of asthma. Second, we evaluated the ability of methylprednisolone to prevent the increase in pulmonary reactivity provoked by propranolol in this asthma model.

Materials and Methods

General Conditions

These studies were approved by the animal research committee of The Johns Hopkins University. The animals used in the study were five BG dogs aged 2–4 yr and weighing 17–23 kg and five mongrel dogs of similar age weighing 19–28 kg. Mongrel and BG dogs were prescreened and selected on the basis of equivalent airway responses in the control state to methacholine. No animal had ever received or was currently receiving any chronic medication. Each dog's response to methacholine was studied under two conditions performed in random order with each study separated by one week. The two conditions were 1) no pretreatment (control) and 2) pretreatment with propranolol (2 mg/kg). After this set of studies, the 5 BG dogs were treated with subcutaneous methylprednisolone (2 mg · kg^{-1} · day^{-1}) for 4 weeks. The studies were then repeated with methacholine challenge under the same two conditions (control and propranolol) in random order on separate days.

The dogs were fasted overnight, were given no preanesthetic medication, and were anesthetized standing, supported in a sling. In all conditions, anesthesia was induced with intravenous thiopental (15 mg/kg), and tracheal intubation was facilitated with succinylcholine (0.5 mg/kg). The dogs' tracheas were intubated with an 8.5-mm cuffed endotracheal tube and the lungs were mechanically ventilated (Harvard Apparatus, Millis, MA)

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with 100% oxygen at a tidal volume of 15 ml/kg and a rate of 15 breaths per min. End-tidal carbon dioxide was sampled continuously using a Perkin Elmer 1100 mass spectrometer (Pomona, CA). Heart rate was continuously monitored with a needle electrode electrocardiogram (Tektronics 412, Beverton, OR) and blood pressure was monitored with an automated blood pressure cuff (Datascope Accutor 1A, Paramus, NJ). After induction, anesthesia was maintained with a continuous intravenous infusion of thiopental (0.2 $mg \cdot kg^{-1} \cdot min^{-1}$) and fentanyl (1 $\mu g/kg$) every 20 min until completion of the study. No additional muscle relaxants were used. Previous studies in this animal model have shown that this anesthetic regimen provides stable and reproducible responses to bronchoconstrictor challenge.

Propranolol was reconstituted from powder with sterile saline to a concentration of 5 mg/ml and administered in a dose of 2 mg/kg over 10 min. This dose was administered with the dogs awake prior to induction. The dose of propranolol was chosen based on previous studies that have demonstrated adequate β-adrenergic blockade based on a standardized isoproterenol sensitivity test.[7]

Measurement of Airway Mechanics

Airflow ($\dot{V}$) was measured using a pneumotachograph (Fleisch type number 1, OEM Medical, Richmond, VA) and a differential pressure transducer (Validyne DP45-16, Northridge, CA), which was connected to one channel of a pen recorder (model 2500S, Gould, Cleveland, OH). A balloon (Spectramed, Dayton, OH) was placed in the esophagus, filled with 1.0 ml of air and withdrawn to the point where end-expiratory pressure was most negative. At the end of each study, correct positioning of the esophageal balloon was verified by the occlusion technique. A second catheter was placed alongside the balloon and connected to suction to keep the esophagus free of air and secretions. Transpulmonary pressure was recorded by connecting one side of a differential pressure transducer (Validyne MP 45-18, Northridge, CA) to the esophageal balloon and the other side to a needle in the airway at the point where the endotracheal tube was connected to the ventilator. The output of the pressure transducer was recorded on the second channel of the pen recorder. Both outputs ($\dot{V}$ and transpulmonary pressure) were electronically processed by a dedicated pulmonary mechanics microprocessor (model 6, Buxco, Sharon, CT) to derive values for lung resistance (R_L) and dynamic compliance (C_{dyn}).[8] Samples were selected at points of the tidal volume cycle to permit a simple solution for the equation relating pressure to flow and volume. Samples were taken at isovolumetric levels both on inspiration and expiration, customarily set to coincide with the instant of maximal expiratory flow. The program ensured that the C_{dyn} was equal at isovolumetric levels of inspiration and expiration. R_L was computed at maximal flow levels. Values for R_L and C_{dyn} were averaged over the six preceding breaths by a computer (model 703, Texas Instruments, Temple, TX). Apparatus resistance (2 $cmH_2O \cdot l^{-1} \cdot s^{-1}$), determined by ventilating a mechanical lung analogy with known parameters, was subtracted from the results to give R_L.

Aerosol Challenges

Thirty minutes after the induction of anesthesia, inhalational challenges with increasing doses of methacholine (0.03, 0.075, 0.15, 0.3, 0.75, and 3.0 mg/ml) were administered. Aerosols were delivered by a Hudson 3000 nebulizer (Hudson, Temecula, CA) driven by compressed oxygen, which delivered aerosol particles with a median mass of 5.7 μm. All solutions were dissolved in distilled water. Aerosol challenges were administered as five standardized breaths using an Ayre's t-tube inserted between the nebulizer and the endotracheal tube. The expiratory port was occluded until an inflation pressure of 15 cmH_2O was obtained. Aerosol challenges were administered at 5-min intervals, and the maximal changes in R_L and C_{dyn} were recorded.

Statistical Analysis

R_L after each challenge was divided by baseline R_L to give the fractional increase in R_L from baseline. C_{dyn} postchallenge was divided by C_{dyn} prechallenge to give the fractional decrease in C_{dyn} from baseline. Responses, defined as an increase in R_L to 200% ($ED_{200}R_L$) and a decrease in C_{dyn} to 65% ($ED_{65}C_{dyn}$) of the prechallenge control value,[9] were calculated for each dog. All data are expressed as the mean ± standard error of the mean (SEM) of five dogs. Changes in R_L and C_{dyn} between BGs and mongrels were analyzed using two-way analysis of variance. When a statistical significance was demonstrated,

TABLE 1. Baseline Values of Pulmonary Resistance and Dynamic Compliance in Basenji-greyhounds and Mongrels

	Basenji-Greyhounds	Mongrels
Resistance ($cm\ H_2O \cdot l^{-1} \cdot s^{-1}$)		
Control	2.55 ± 0.12	2.43 ± 0.14
Propranolol	2.40 ± 0.18	2.32 ± 0.20
Steroids	2.47 ± 0.24	—
Steroids + propranolol	2.53 ± 0.21	—
Compliance ($ml \cdot cmH_2O^{-1}$)		
Control	64 ± 4.2	66 ± 5.4
Propranolol	68 ± 6.8	64 ± 7.9
Steroids	66 ± 4.9	—
Steroids + propranolol	63 ± 7.2	—

Measurements made prior to the start of aerosol methacholine challenges and after the administration of propranolol. Each value is the mean ± SEM of five dogs.

matched means were compared using the method of least significant difference. $ED_{200}R_L$ and $ED_{65}C_{dyn}$ were compared using paired and unpaired t tests with the Bonferroni adjustment when multiple comparisons were performed.

Results

No significant difference was seen in the baseline measurements of R_L and C_{dyn} between the mongrels and the BG dogs prior to the start of aerosol challenge (table 1). Propranolol did not significantly change baseline R_L or C_{dyn} in either the BG dogs or mongrels (table 1). Furthermore, neither methylprednisolone nor propranolol altered baseline R_L or C_{dyn} in the BG dogs (table 1).

There was no significant difference between the BG dogs and mongrels in the pulmonary responses to methacholine in the control state (fig. 1). Methacholine produced dose-dependent increases in R_L and decreased in C_{dyn} in both BG dogs and mongrels. The pulmonary responses to methacholine were significantly greater ($P < 0.05$) at all concentrations of methacholine in the BG dogs after pretreatment with propranolol than in the control state (fig. 2). In BG dogs, the $ED_{200}R_L$ was 0.17 ± 0.03 mg/ml in the control state and decreased to 0.05 ± 0.02 mg/ml ($P < .05$) after pretreatment with propranolol (table 2). The $ED_{65}C_{dyn}$ was 0.53 ± 0.17 mg/ml and decreased to 0.07 ± 0.01 mg/ml ($P < 0.01$) after pretreatment with propranolol (table 3). Propranolol did not significantly change pulmonary reactivity to methacholine in the mongrels (fig. 3). In mongrel dogs the $ED_{200}R_L$ was 0.20 ± 0.05 mg/ml in the control state and 0.18 ± 0.04 mg/ml after pretreatment with propranolol, (table 2) while the $ED_{65}C_{dyn}$ was 0.61 ± 0.21 mg/ml and 0.58 ± 0.19 mg/ml, respectively, for these two conditions (table 3).

Methylprednisolone administration itself did not change pulmonary responses in the BG dogs (tables 2 and

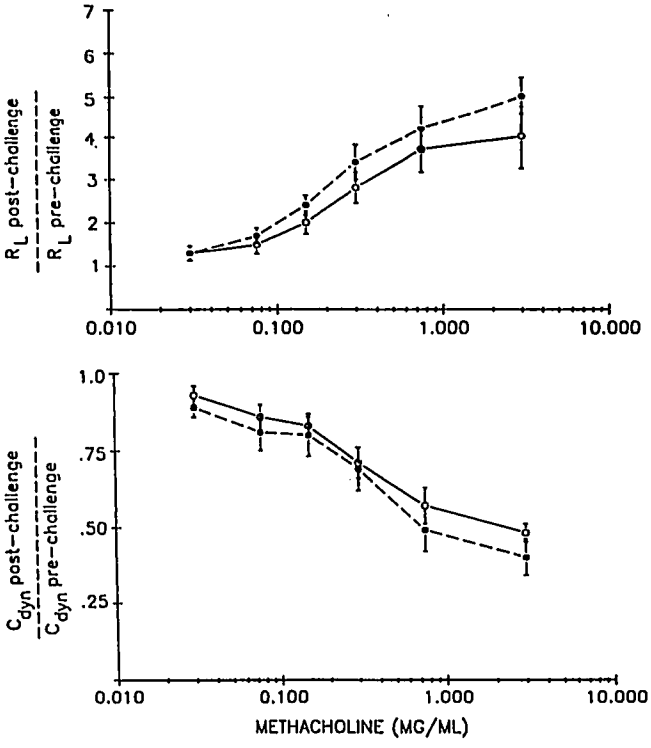

FIG. 1. Dose–response curves to aerosol methacholine challenge in BG dogs (solid line) and mongrel dogs (dashed line). Pulmonary resistance (R_L) above and dynamic compliance (C_{dyn}) below. Each value is the mean ± SEM of five dogs.

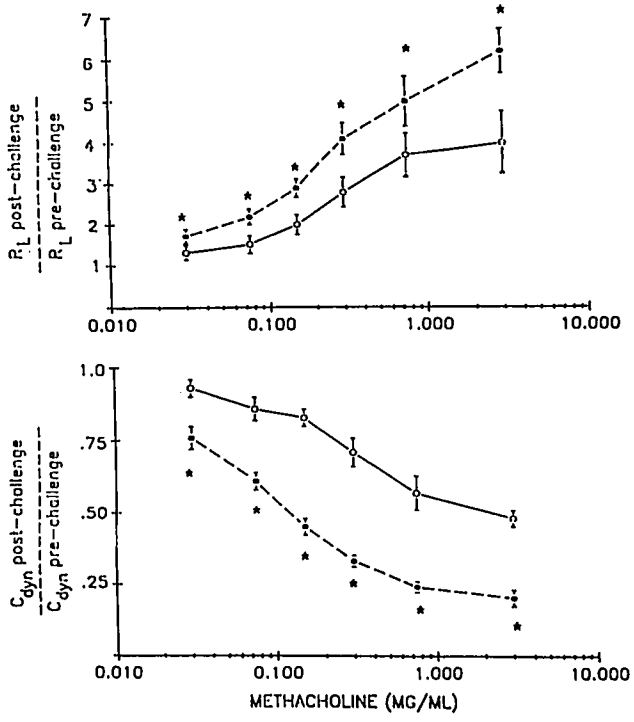

FIG. 2. Dose–response curves to aerosol methacholine challenge in BG dogs with (dashed line) and without propranolol (solid line). Pulmonary resistance (R_L) above and dynamic compliance (C_{dyn}) below. Each value is the mean ± SEM of five dogs. Those values that are significantly different ($P < 0.05$) are marked with an asterisk(*).

TABLE 2. $ED_{200}R_L$ of Methacholine in Basenji-greyhounds and Mongrels

	Basenji-greyhounds (mg/ml)	Mongrels (mg/ml)
Control	0.17 ± 0.03	0.20 ± 0.05
Propranolol	0.05 ± 0.02*	0.18 ± 0.04
Steroids	0.16 ± 0.03	—
Steroids + propranolol	0.22 ± 0.06	—

Each value is the mean ± SEM of five dogs.
* $P < 0.05$ when compared to all other groups.

TABLE 3. $ED_{65}C_{dyn}$ of Methacholine in Basenji-greyhounds and Mongrels

	Basenji-greyhounds (mg/ml)	Mongrels (mg/ml)
Control	0.53 ± 0.17	0.61 ± 0.21
Propranolol	0.07 ± 0.01*	0.58 ± 0.19
Steroids	0.58 ± 0.14	—
Steroids + propranolol	0.59 ± 0.23	—

Each value is the mean ± SEM of five dogs.
* $P < 0.05$ when compared to all other groups.

3). However, in BG dogs receiving chronic methylprednisolone, propranolol no longer increased the pulmonary responses to methacholine (fig. 4 and tables 2 and 3). In methylprednisolone-treated BG dogs in the absence of propranolol, the $ED_{200}R_L$ was 0.16 ± 0.03 mg/ml and the $ED_{65}C_{dyn}$ was 0.58 ± 0.14 mg/ml. After propranolol administration in methylprednisolone-treated BG dogs, the $ED_{200}R_L$ was 0.22 ± 0.06 mg/ml and the $ED_{65}C_{dyn}$ was 0.59 ± 0.23 mg/ml.

Discussion

This study demonstrates the difference in the effects on airway reactivity of the β-adrenergic antagonist, propranolol, between BG and mongrel dogs. Although propranolol did not significantly alter airway reactivity to methacholine aerosol challenge in the mongrels, it significantly increased airway reactivity in the BGs. The propranolol-induced airway hyperreactivity to methacholine was no longer present after 4 weeks of corticosteroid treatment in the BG dogs. Possible mechanisms to explain these findings include a property of β-adrenergic antagonists unrelated to their ability to block β-receptors, unopposed vagal stimulation, different levels of sympathetic tone in the two breeds of dogs, or a defect in signal transduction at the level of the airway smooth muscle cell.

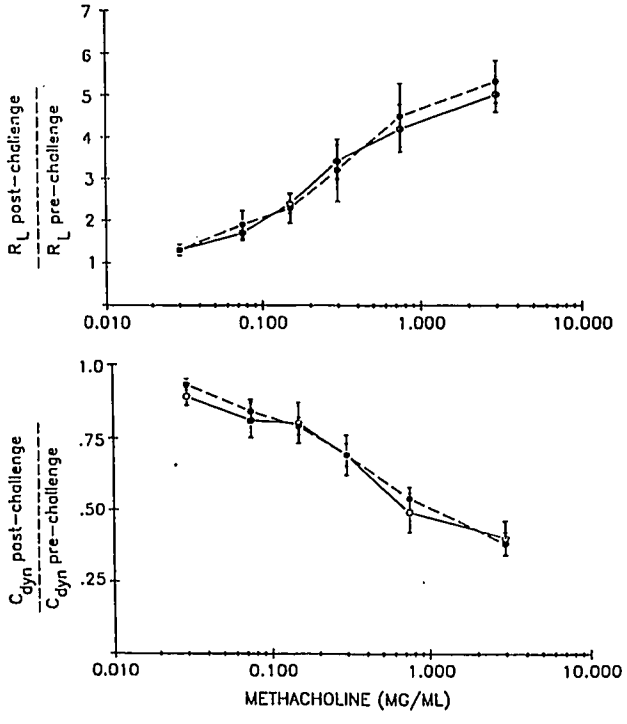

FIG. 3. Dose–response curves to aerosol methacholine challenge in mongrel dogs (dashed line) and without propranolol (solid line). Pulmonary resistance (R_L) above and dynamic compliance (C_{dyn}) below. Each value is the mean ± SEM of five dogs.

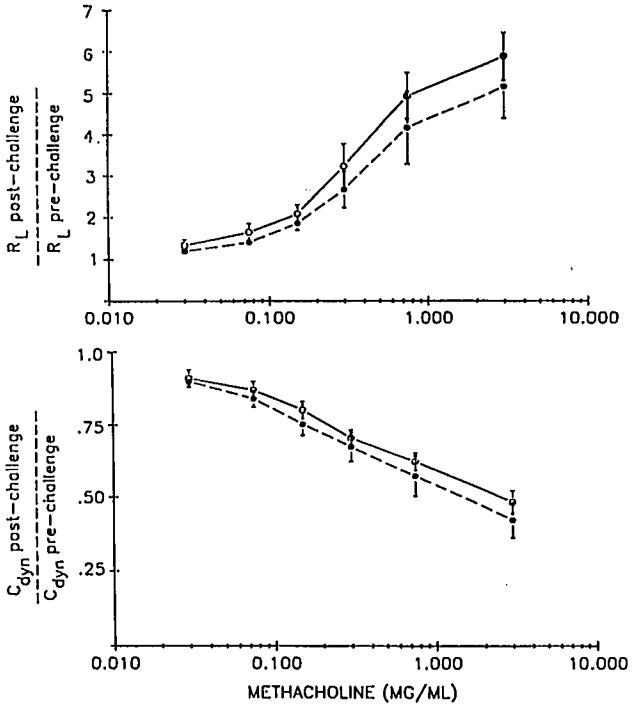

FIG. 4. Dose–response curves to aerosol methacholine challenge in BG dogs with (dashed line) and without (solid line) propranolol after 2 weeks of methylprednisolone. Pulmonary resistance (R_L) above and dynamic compliance (C_{dyn}) below. Each value is the mean ± SEM of five dogs.

It is unlikely that differing levels of vagal tone between BG and mongrel dogs explain our results. Propranolol-induced potentiation of airway responses is only partially blocked by muscarinic antagonists such as atropine[10] and oxitropium,[11] and this phenomenon persists after atropine or vagotomy.[12]

It is also unlikely that the differences between BGs and mongrels resulted from differing levels of sympathetic tone. We have previously demonstrated that circulating epinephrine and norepinephrine levels in the basal state during methacholine challenge and in response to aminophylline infusion were similar in BG and mongrel dogs.[13]

Blockade of β_2-adrenergic receptors appears to be involved in propranolol-induced airway hyperreactivity since the L-isomer of propranolol causes bronchoconstriction, whereas the D-isomer, which is devoid of significant β-receptor blocking activity, does not.[14] Moreover, a β_1-selective antagonist (esmolol), in doses producing equipotent β_1-blocking effects, does not potentiate methacholine-induced bronchoconstriction in BG dogs.[15] However, the site in the β-adrenergic cascade that differs in BG and mongrels and perhaps in asthmatic and nonasthmatic subjects is unclear. The inability of β-adrenergic antagonists to produce asthma in normal individuals after β-adrenergic blockade is often cited as evidence against a β-adrenergic defect in asthma. However, this suggests only that the defect may exist distal to the receptor itself. Rather, the consistent worsening of asthma by β-adrenergic antagonist administration illustrates the importance of the β-adrenergic mechanism in maintaining airway tone in patients with asthma.

Snapper *et al.*[16] found no significant potentiation by propranolol of either histamine or prostaglandin $F_{2\alpha}$ reactivity in mongrel dogs. We are unaware of analogous studies using cholinergic reactivity as an index in mongrel dogs. It is unlikely that propranolol potentiated methacholine responses by increasing basal tone, as propranolol did not increase baseline tone in this study. In a study in BG dogs published in 1981,[17] a small increase in airway tone by propranolol made interpretation of the results difficult. We have no explanation for the differing effects of propranolol on baseline tone in the two studies except that the five dogs in each study were not the same dogs and were raised in different environments. The dogs in the 1981 study[17] were more responsive to methacholine than were the dogs in the current study, which were selected to match the methacholine reactivity of the mongrels.

There are several mechanisms by which glucocorticoids may act on the airways to prevent propranolol-induced potentiation of methacholine reactivity. Corticosteroids may reduce inflammation and the liberation of inflammatory mediators that increases airway reactivity by altering airway geometry.[18] Glucocorticoids are also known to specifically alter β-adrenergic function. Glucocorticoids increase the expression of β-adrenergic receptors by altering rates of receptor synthesis and degradation.[5] Recently, dexamethasone was shown to increase transcription of the β-adrenergic receptor gene and to increase steady-state levels of β-adrenergic receptor messenger ribonucleic acid (mRNA).[19] However, glucocorticoid-induced upregulation of β-adrenergic function should potentiate, not prevent, propranolol-induced increases in methacholine reactivity, suggesting that perhaps our current knowledge of these intracellular pathways is incomplete. In addition, glucocorticoids can modulate levels of mRNA encoding the G-protein subunits that link the β-adrenergic receptors[5] and perhaps other receptors to their intracellular second messengers.

Four weeks of glucocorticoid therapy had no effect on methacholine reactivity in either this study or in a previous[20] study in BG dogs but did significantly alter β-adrenergic responses.[21] Although this study shows that corticosteroids given for 4 weeks produce significant effects, it is possible that shorter courses also may be effective. Significant effects of corticosteroids on airways are found at 24 h and at 3 days,[22] and 1 week of corticosteroid treatment restored β-adrenergic responsiveness in our model.[23] Although the dose of steroid chosen for these studies was large, it was administered only once per day and is therefore within the range of doses used clinically (0.5–1.0 mg/kg every 6 h).

We conclude from this study that chronic corticosteroid therapy blocks the increased airway reactivity to methacholine caused by propranolol in the BG dog model of asthma. As always, caution should be exercised when applying results obtained in laboratory animals to clinical practice; however, these findings suggest that chronic corticosteroid treatment may prevent the adverse pulmonary effects of β-adrenergic antagonists when clinical care dictates the use of β-antagonists in patients with reactive airway disease. However, further studies are needed to define the effective dose and duration of corticosteroid therapy needed with asthma and COPD.

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