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Comparison of Drug Responses *in Vivo* and *in Vitro* in Airways of Dogs with and without Airway Hyperresponsiveness[1]

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ABSTRACT

Basenji-greyhound (BG) dogs demonstrate marked nonspecific airway hyperresponsiveness. To assess the possible contribution of an abnormal sensitivity of airway smooth muscle to this phenomenon, we studied the *in vitro* contractile responses to methacholine and histamine and the relaxant response to isoproterenol in trachealis muscle from five BG dogs with airway hyperresponsiveness *in vivo* and from five greyhound dogs that served as a control population. Isoproterenol responses were determined against a half-maximal methacholine contraction. Aerosol methacholine concentrations required to produce a 2-fold increase in pulmonary resistance were 0.07 ± 0.02 (±S.E.) mg/ml in BG dogs and 0.67 ± 0.26 mg/ml in greyhounds; pD_2 values for methacholine-induced contraction of cervical trachealis muscle were 7.03 ± 0.11 in BG dogs and 7.50 ± 0.11 in greyhounds. A significant ($P < .01$) negative correlation was found between methacholine sensitivity *in vivo* and *in vitro*. Aerosol concentrations of histamine required to produce a 2-fold increase in pulmonary resistance were 0.19 ± 0.06 mg/ml in BG dogs and 1.44 ± 0.43 mg/ml in greyhounds; pD_2 values for histamine were identical in BG dogs (4.95 ± 0.08) and greyhounds (5.05 ± 0.19). Isoproterenol pD_2 values were less in the trachealis muscle (cervical) of BG dogs (6.76 ± 0.10) than in that of greyhounds (7.93 ± 0.16), but this is probably a consequence of the higher concentration of methacholine needed to contract BG muscles. We conclude that the airway hyperresponsiveness of BG dogs does not reflect an increased sensitivity of airway smooth muscle *per se*.

In the search for the defect underlying asthma, mechanical and biochemical properties of airway smooth muscle have been compared in unsensitized and antigen-sensitized animals of several species. Although differences in drug responses have been found (Souhrada, 1978; Antonissen *et al.*, 1979; Rubinfeld *et al.*, 1982; Kaukel *et al.*, 1984; Morcillo *et al.*, 1984), these studies are difficult to interpret because the animals lacked the nonspecific airway hyperresponsiveness characteristic of human asthma, and the observed differences may have reflected changes associated with allergy and the process of sensitization rather than asthma.

In contrast to most animal models of asthma, the BG crossbred dog does demonstrate nonspecific airway hyperresponsiveness to a variety of aerosol challenges including methacholine (Hirshman *et al.*, 1980) and calcium chelators (Downes and Hirshman, 1983). As with asthmatic patients, BG dogs frequently present evidence of concomitant allergy (Butler *et al.*, 1983; Hirshman *et al.*, 1984), but airway hyperresponsiveness and allergic manifestations are not closely linked. To determine if abnormal smooth muscle responses are the cause of the *in vivo* hyperresponsiveness of BG dogs, we studied the mechanical responses to methacholine, histamine and isoproterenol in isolated trachealis muscle preparations of five BG and five unrelated greyhounds, which had been tested previously for sensitivity to methacholine and histamine aerosols *in vivo*.

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Methods

Subjects. The studies were performed in five BG dogs and five unrelated greyhounds that were age matched (~2 years old) and housed under identical conditions in the animal quarters at Oregon Health Sciences University. BG dogs ranged from 18 to 23 kg and greyhounds from 27 to 37 kg. All animals had been selected especially for this study, and had not been tested previously as part of other ongoing studies.

Pulmonary mechanics. The dogs were not premedicated and were anesthetized standing supported by a sling. After induction of anesthesia with i.v. thiopental (12–15 mg/kg) the dogs were paralyzed with succinylcholine (0.5 mg/kg), intubated with a 8.5 to 9.0 mm cuffed endotracheal tube and ventilated mechanically (Harvard Apparatus, Millis, MA) with 100% O_2 at a tidal volume of 15 ml/kg and a frequency of 15/min. Additional increments of thiopental (2 mg/kg) and succinylcholine (0.2 mg/kg) were administered as needed at ~20-min intervals. An esophageal balloon (Dynasciences, Blue Bell, PA) was placed in the esophagus and positioned at the point where recorded end-expiratory pressure was lowest. The balloon contained 0.8 to 1.5 ml of air. A separate catheter connected to suction was placed in the esoph-

ABBREVIATIONS: BG, basenji-greyhound; P_L, transpulmonary pressure; C_{dyn}, dynamic compliance; R_L, pulmonary resistance.

agus to keep it empty of air and liquid. P_L was measured with a differential transducer (Hewlett-Packard 270, Waltham, MA) connected to the esophageal balloon and to a needle inserted into the endotracheal tube. Airflow ($\dot{V}$) was measured with a pneumotachograph head (Hewlett-Packard 2100) and a differential flow transducer (Hewlett-Packard 47304A). Pressure and flow signals were recorded with a Hewlett-Packard 47601 polygraph. C_{dyn} was calculated by dividing tidal volume by the difference in pressure between points of zero flow. R_L was calculated using the method of von Neergaard and Wirz (1927) by dividing P_L minus elastic pressure by $\dot{V}$ at midtidal volume. Apparatus resistance (1.5–2.5 $cmH_2O \cdot l^{-1} \cdot sec$), determined by ventilating a mechanical lung analog with known parameters, was subtracted from the resulting value to give R_L. C_{dyn} and R_L were calculated from a mean of five consecutive breaths.

Aerosol challenges. Aerosols were administered with a Hudson 3000 nebulizer (Hudson, Temecula, CA) driven by compressed O_2, which delivered aerosol particles with a mass median diameter of 5.7 μm. All aerosol solutions were made up in distilled water. Methacholine and histamine aerosols were administered for five breaths, using an Ayre's T tube inserted between the nebulizer and the endotracheal tube (Hirshman *et al.*, 1980, 1984). The expiratory port of the T tube was occluded until an inflation pressure of 15 cmH_2O had been obtained for each breath. Peak changes in R_L and C_{dyn} were observed within the first 2-min postchallenge. Increasing drug concentrations were administered at ~10-min intervals, which was sufficient to allow return of R_L and C_{dyn} to control values between challenges. The concentrations needed to produce a 2-fold increase in R_L (ED_{200} R_L) and a decrease in C_{dyn} to 65% of control values (ED_{65} C_{dyn}) were extrapolated from log dose-response curves using 4 to 7 doses/animal. Aerosol methacholine bromide concentrations were 0.03, 0.075, 0.15, 0.30, 0.75, 1.5 and 3.0 mg/ml; histamine diphosphate concentrations were 0.03, 0.1, 0.3, 1.0, 3.0 ml and 10 mg/ml. *Ascaris suum* antigen was prepared as described previously (Hirshman *et al.*, 1980) and contained 30 μg of *Ascaris* protein in 10 ml of water. *Ascaris* antigen aerosols were administered for a 5-min period by manual ventilation with the nebulizer inserted between the inspiratory limb of a circle anesthesia system and the endotracheal tube (Hirshman *et al.*, 1980). Each challenge aerosol was delivered in a separate experiment about 1 week apart and within 6 weeks of sacrifice.

Trachealis muscle. The dogs were sacrificed under halothane anesthesia by radical excision of trachea, lungs and heart. Portions of lung and bronchi were fixed or frozen for a variety of anatomical and biochemical studies that were not directly related to the present experiments. The trachea was immediately placed in cold (4°C), aerated (95% O_2-5% CO_2) Krebs-Henseleit solution of the following composition (millimolar): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11.1. Less than 5 min elapsed between circulatory arrest (ligature occlusion of the great vessels of the heart) and insertion of the excised trachea in the Krebs-Henseleit solution. The trachea was divided into cervical and thoracic segments, and only tissue from the upper and middle cervical and lowermost thoracic portions was used for this study. Small strips of trachealis muscle (~0.5 × 2.5 × 10 to 20 mm) were dissected free of epithelium and connective tissue, and mounted in 50-ml organ baths containing aerated Krebs-Henseleit solution and maintained at 37°C. After completion of an experiment, preparations were blot-dried and weighed. Muscle strips from BG and greyhounds average 29 ± 2 and 55 ± 4 mg (±S.E.), respectively. All muscles were cut to as near the same width as possible, and the greater weight of the greyhound muscles reflected their longer length.

Because previous studies (G. A. Rinard, personal communication; Souhrada *et al.*, 1983) have disclosed large differences in drug responses of trachealis muscle, depending upon its position within the trachea, most of the present work used trachealis muscle from two different anatomic sites, that should be strictly comparable in BG and greyhound dogs: The uppermost trachea, just below the cricoid cartilage, and the lowermost trachea, just above the carina. These two areas will be subsequently referred to as "cervical" and "thoracic" trachealis muscle. Middle cervical trachealis muscle, just distal to the cervical samples described above, was used for studies of the contractile response to histamine.

Trachealis muscle strips were mounted for isotonic recording (Harvard Apparatus) under 4 g of tension and at gain of ~2.5 times. The preparations were washed three times with fresh Krebs-Henseleit solution immediately after mounting and then at 15-min intervals for an initial stabilization period of ~1 hr before determining dose-response curves for methacholine- or histamine-induced contraction. Cumulative dose-response curves were obtained by increasing drug concentration in half-log units from 10^{-9} to 10^{-4} M for methacholine, and from 10^{-7} to 10^{-3} M for histamine. The concentration (molar) that produced a half-maximal effect (ED_{50}) was found as the negative antilog of the pD_2 ($pD_2 = -\log ED_{50}$), which was determined from Hill plot regression lines using points that fell between 10 to 90% of maximal effect. Each preparation was used only once to determine a single dose-response curve. Because the maximal effect of histamine is less than that of methacholine, preparations used to study histamine effects were subsequently contracted maximally with methacholine.

Inasmuch as canine trachealis muscle has little or no intrinsic tone, the relaxant effect of isoproterenol was studied in preparations that had been contracted with an ED_{50} of methacholine (fig. 1), as individually determined in the next adjacent strip of trachealis muscle. Therefore, isoproterenol dose-response curves were determined after completion of the methacholine dose-response curve in the adjacent strip, at ~2 to 3 hr after mounting. All chambers used to test the effect of isoproterenol contained 10^{-4} M ascorbic acid. Isoproterenol concentration was increased in half-log units from 10^{-9} to 10^{-4} M. In cervical preparations (fig. 1), the maximal effect of isoproterenol usually resulted in complete, or nearly complete, relaxation of the methacholine contraction; however, in thoracic strips, the maximal effect of isoproterenol was something less than complete relaxation. This was expressed as the ratio of the maximal effect of isoproterenol (E_{max} isoproterenol) to full relaxation of the methacholine contraction (fig. 6). Ratios slightly greater than unity were obtained in a few cervical preparations, indicating a slight relaxation over and above complete reversal of the methacholine contraction.

Statistical analyses. Data are expressed as the mean ±S.E. Differences between means from BG and greyhound dogs were compared by the unpaired Student's *t* test. Differences between cervical and thoracic preparations from the same animals were compared using the paired Student's *t* test.

Drugs. Ascorbic acid, histamine diphosphate, racemic isoproterenol hydrochloride and methacholine bromide were obtained from Sigma Chemical Company (St. Louis, MO). All drugs used for *in vitro* studies were dissolved in distilled water and added to the organ baths in

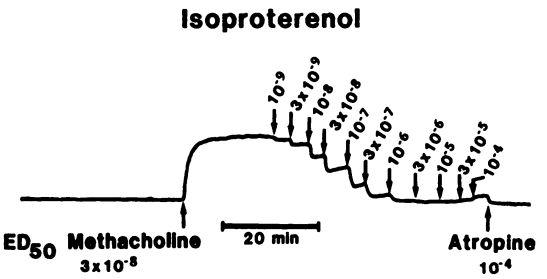

Fig. 1. Isoproterenol-induced relaxation of cervical trachealis muscle. Muscle was contracted initially with a concentration of methacholine that produced a half-maximal contraction, as determined in an adjacent muscle strip; when the contraction had plateaued, isoproterenol was added in cumulative half-log increments. Addition of drug is indicated by an arrow with the cumulative drug concentration (molar) appended. The trachealis muscle is from a greyhound dog and demonstrates complete relaxation of the methacholine-induced contraction typical of cervical preparations. Subsequent addition of atropine elicited no further relaxation than the maximal effect of isoproterenol alone.

increments of 0.5 ml or less. Ascorbic acid (10^{-4} M) was present in all solutions containing isoproterenol.

Results

Aerosol challenges. Mean values for the effective aerosol concentrations of methacholine and histamine were about an order of magnitude lower in BG than in greyhound dogs (table 1). One greyhound was considerably more sensitive than its fellows, and responded to concentrations at the upper limit of those effective in BG dogs. Effective concentrations in this greyhound were (milligrams per milliliter): methacholine, ED_{200} R_L 0.13, ED_{65} C_{dyn} 0.15; histamine, ED_{200} R_L 0.4, ED_{50} C_{dyn} 0.5.

Neither BG nor greyhounds had been sensitized by intentional exposure to antigen. On challenge with aerosols of *Ascaris* antigen, one greyound showed a 1.8-fold increase in R_L and a decrease in C_{dyn} to 84% of control, and one BG showed a 2.3-fold increase in R_L and a decrease in C_{dyn} to 67% of control; the remaining animals were unresponsive to *Ascaris* antigen.

Trachealis muscle, methacholine. Thoracic trachealis muscle was significantly ($P < .01$) less sensitive than cervical trachealis muscle obtained from the same animals, and muscle from BG dogs was significantly ($P < .05$) less sensitive than comparable tissue from greyhounds (fig. 2; table 2). In individual experiments, the difference in methacholine pD_2 between cervical and thoracic preparations ranged from 0.77 to 1.42 (mean 1.12). Because there was a close correlation ($r = 0.96$) between the pD_2 values obtained in cervical and thoracic preparations, the relative order of sensitivity between animals was similar in both regions, despite the large inter-regional differences in pD_2.

The maximal effect of methacholine, as measured by millimeters of contraction was significantly ($P < .05$) less in comparable preparations from BG as compared to greyhound dogs (table 2). When maximal contraction was normalized in terms of milligrams of muscle (to compensate for the greater length of preparations obtained from greyhounds), there was no significant difference between comparable preparations from BG and greyhound dogs. The quotients (millimeters of contraction per milligram of muscle weight) were 0.41 ± 0.04, 0.30 ± 0.06, 0.30 ± 0.06 and 0.27 ± 0.02 in BG cervical, greyhound cervical, BG thoracic and greyhound thoracic preparations, respectively.

In vivo potency as determined by the log ED_{200} R_L, showed a significant ($P < .05$) negative correlation with *in vitro* potency, as determined by the log ED_{50}, both in cervical (fig. 3) ($r = -0.63$) and thoracic ($r = -0.71$) preparations. *In vivo* potency as determined from the log ED_{65} C_{dyn} also showed a significant negative ($P < .05$) correlation with *in vitro* potency, both in cervical ($r = -0.70$) and thoracic ($r = -0.76$) preparations. Muscle from the greyhound that was most responsive *in vivo* (see above) was the least responsive *in vitro*; values of methacholine pD_2 in cervical and thoracic trachealis muscle from this greyhound were 7.08 and 5.65, respectively, and comparable to the average values in BG dogs (table 2).

Trachealis muscle, histamine. Values of pD_2 determined for histamine in middle cervical trachealis muscle were virtually identical in both BG (pD_2 ± S.E. = 4.95 ± 0.08) and greyhound dogs (5.05 ± 0.19). After completion of the histamine dose-response curves, these preparations were washed and contracted maximally with methacholine. The maximal effect of histamine in both types of dog was consistently less than that of methacholine. Although the maximal effect of histamine relative to methacholine was less in the BG dogs (41 ± 5% of maximal contraction) than in the greyhounds (55 ± 13% of maximal contraction), this difference was not significant.

In contrast to methacholine, the *in vitro* log ED_{50} histamine showed no correlation with *in vivo* measurements of ED_{200} R_L (fig. 4, $r = -0.26$) or ED_{65} C_{dyn} ($r = -0.13$).

Trachealis muscle, isoproterenol. When isoproterenol was tested against the ED_{50} of methacholine, there were significant differences between regions and between dogs, with respect to both the pD_2 and the maximal effect (table 3). Both the pD_2 and maximal effect of isoproterenol were significantly ($P < .01$) greater in cervical as contrasted to thoracic preparations from the same animals, and significantly ($P < .01$) greater in comparable preparations obtained from greyhounds as opposed to BG dogs. However, the concentrations of methacholine required to elicit the test contraction showed similar differences between regions and type of dog (see above), and there was a significant correlation ($P < .01$) between the ED_{50} of methacholine (used to elicit the test contraction) and the isoproter-

TABLE 1

Effective concentrations (milligrams per milliliter) during aerosol challenge

Means ± S.E., five BG and five greyhounds.

Methacholine	ED_{200} R_L	ED_{65} C_{dyn}
BG	0.07 ± 0.02	0.06 ± 0.01
Greyhound	0.67 ± 0.26	0.68 ± 0.28
Histamine	**ED_{200} R_L**	**ED_{65} C_{dyn}**
BG	0.19 ± 0.06	0.31 ± 0.12
Greyhound	1.44 ± 0.43	2.56 ± 1.07

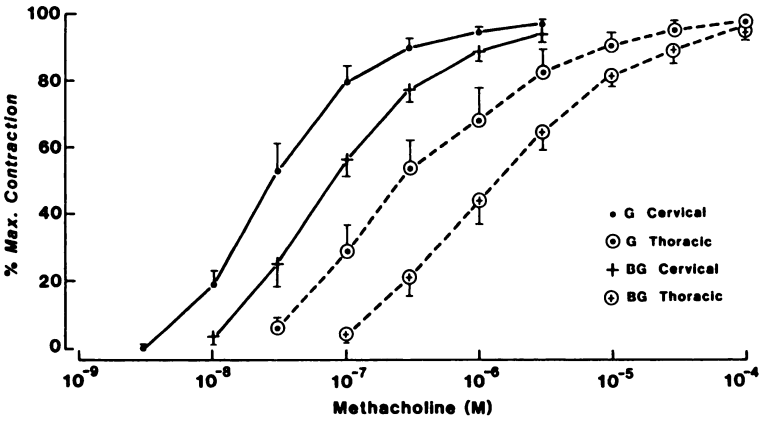

Fig. 2. Cumulative dose-response curves for methacholine-induced contraction in cervical and thoracic trachealis muscle from BG and greyhound (G) dogs. Cervical strips are ~ an order of magnitude more sensitive than thoracic strips for both types of dog. Methacholine was less potent in BG dogs in both cervical and thoracic preparations ($P < .05$). Max, maximum.

TABLE 2

***In vitro* response to methacholine**

Mean ± S.E. Numbers in parentheses, number of muscles.

	pD_2[a]		E_{max}[b]	
	BG	Greyhound	BG	Greyhound
Cervical trachealis muscle	7.03 ± 0.11 (5)	7.50 ± 0.11 (5)	12.3 ± 1.8 (5)	16.3 ± 1.8 (5)
Thoracic trachealis muscle	5.85 ± 0.14 (4)	6.48 ± 0.22 (4)	7.9 ± 0.8 (4)	12.4 ± 1.25 (4)

[a] −log ED_{50}.
[b] Millimeter of contraction.

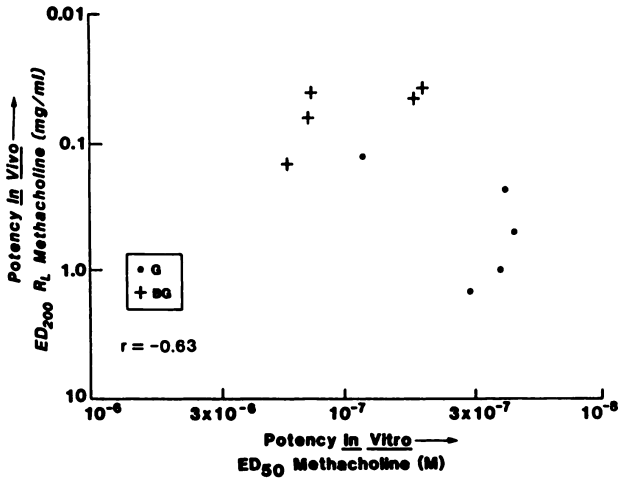

Fig. 3. Methacholine sensitivity *in vivo* and *in vitro*. The log ED_{200} R_L is plotted on the vertical axis and the log ED_{50} of methacholine for contraction of the upper cervical trachealis muscle is plotted on the horizontal axis. Values are inversely correlated ($r = -0.63$). BG dogs were more sensitive than greyhounds (G) *in vivo*, but their trachealis muscle was less sensitive *in vitro* . Note that the one hyperresponsive G *in vivo* also produced a hyporesponsive trachealis preparation.

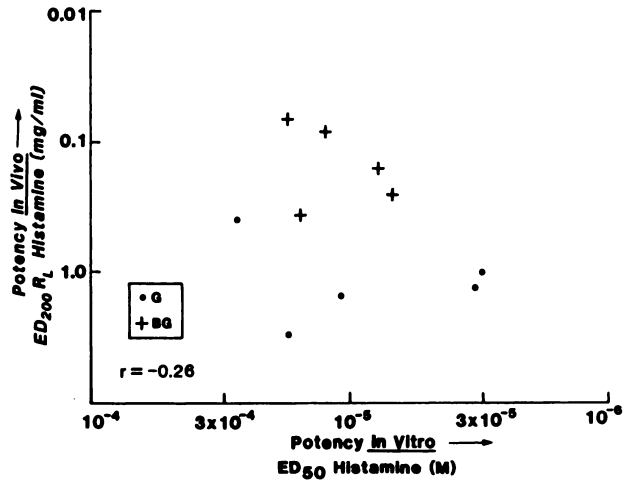

Fig. 4. Lack of correlation between histamine sensitivity *in vivo* and *in vitro*. The log ED_{200} R_L is plotted on the vertical axis and the log ED_{50} of histamine for contraction of middle cervical trachealis muscle is plotted on the horizontal axis. G, greyhound.

enol ED_{50} (fig. 5) ($r = 0.83$). Similarly, there was a significant negative correlation ($P < .01$) between the ED_{50} of methacholine and the maximal effect of isoproterenol (fig. 6; $r = -0.75$). Potency and efficacy of isoproterenol were greatest in the cervical trachealis muscle of greyhounds and least in the thoracic trachealis muscle of BG dogs; these were the preparations that required the lowest and highest concentrations of methacholine, respectively. Methacholine doses used to elicit the test contraction in BG cervical trachealis muscle and greyhound thoracic trachealis muscle showed considerable overlap (fig. 5), and the values for pD_2 and maximal effects in these two groups were virtually identical (table 3, diagonal arrows).

Discussion

The BG dog model of asthma reproduces many characteristics of the human disease (Hirshman, 1985), including nonspecific airway hyperresponsiveness. Such hyperresponsiveness presumably can occur as a statistical extreme in the mongrel dog population (Snapper *et al.*, 1978), and one of the greyhounds used in the present study also was relatively hyperresponsive both with respect to its fellows and with respect to most mongrel dogs as tested in our laboratory (Hirshman *et al.*, 1980). However, among BG dogs, airway hyperresponsiveness is the norm rather than the exception. Such hyperresponsiveness could reflect one or several of the following general mechanisms: enhanced access of challenge substances to receptors on airway smooth muscle or related structures, enhanced effects independent of access factors, a deficit in *beta* adrenergic or other inhibitory control systems, enhanced vagal responses or an increased synthesis (or decreased catabolism) of spasmogenic chemical mediators. Previous studies have failed to demonstrate increased permeability of the bronchial mucosa in BG compared to mongrel dogs (Taylor *et al.*, 1983), but did show a blunted cyclic nucleotide response to isoproterenol stimulation in leukocytes from BG dogs (Chan *et al.*, 1985) and release into blood of a leukotriene-type mediator during the bronchoconstriction induced by calcium chelators in BG dogs (Hirshman *et al.*, 1983a,b). Vagal reflexes appear to play little role in the airway hyperresponsiveness of BG dogs as large doses of atropine (0.2 mg/kg i.v.), that were adequate to block completely the increase in R_L evoked by supramaximal stimulation of the vagus nerve (Hirshman and Downes, 1981), did not alter the marked bronchoconstrictor response to calcium chelators (Hirshman *et al.*, 1983b; Downes and Hirshman, 1983) and only slightly diminished the equally marked response to *Ascaris* antigen aerosols in previously sensitized animals (Hirshman and Downes, 1981). *Ascaris*-induced increases in R_L in such atropine-pretreated BG dogs were several-fold greater than responses in mongrel dogs without atropine pretreatment.

The *in vitro* responses of airway smooth muscle from BG dogs have not been studied previously. An apparent increase in agonist potency or efficacy in isolated airway smooth muscle could explain, at least in part, the marked hyperresponsiveness observed in the intact animal and would provide direction for future studies. Conversely, lack of any difference between ago-

TABLE 3

***In vitro* response to isoproterenol**

Mean ± S.E. Numbers in parentheses, number of muscles.

	pD_2[a]		E_{max}[b]	
	BG	Greyhound	BG	Greyhound
Cervical trachealis muscle	6.75 ± 0.10 (5)	7.93 ± 0.16 (5)	78 ± 8% (5)	102 ± 1% (5)
Thoracic trachealis muscle	5.94 ± 0.21 (4)	6.80 ± 0.20 (4)	52 ± 7% (4)	75 ± 15 (4)

[a] −log ED_{50}.

[b] Maximal relaxation achieved by isoproterenol as percentage of full relaxation of the methacholine ED_{50} contraction.

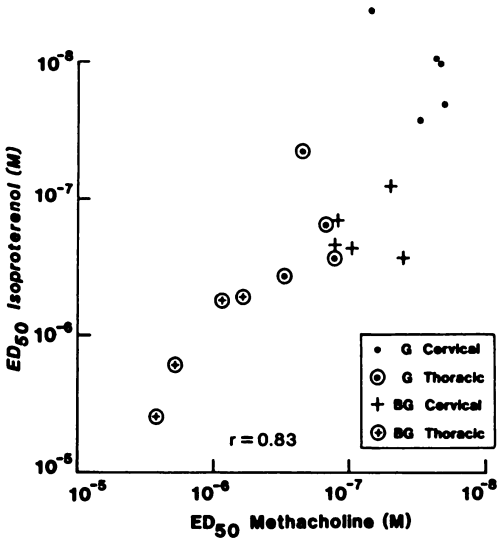

Fig. 5. Correlation (r = 0.83) of log ED_{50} of isoproterenol and of methacholine in cervical and thoracic trachealis muscle from BG and greyhound (G) dogs. The concentration of methacholine needed to produce a half-maximal contraction in an adjacent strip was used to precontract the trachealis muscle before addition of isoproterenol. BG muscles that required higher concentrations of methacholine, also required higher concentrations of isoproterenol. Note the area of overlap in the middle of the line, composed of cervical trachealis muscle from BG dogs and thoracic trachealis from G dogs; these preparations, which received ~ equal concentrations of methacholine to elicit a test contraction, were equally sensitive to isoproterenol.

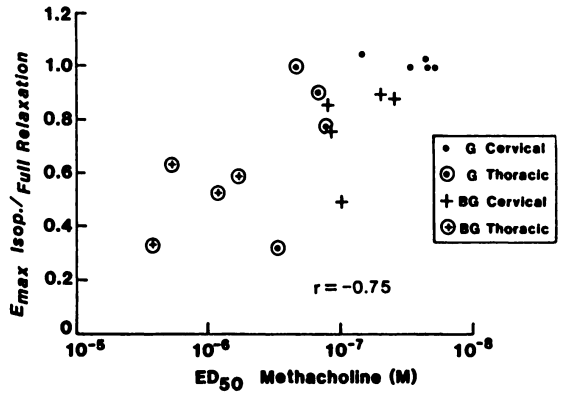

Fig. 6. Negative correlation (r = −0.75) of the log ED_{50} methacholine (test contraction) and the maximal effect [E_{max} isoproterenol (Isop)] of Isop. Isop had the least effect in those preparations that required the highest concentrations of methacholine to elicit the test contractions (BG thoracic trachealis muscle); conversely, greyhound (G) cervical trachealis muscle, which required much lower concentrations of methacholine to produce the test contractions, were fully relaxed by isop.

nist responses in airway smooth muscle *in vitro* would suggest that the defect responsible for hyperresponsiveness *in vivo* lay elsewhere than in the pharmacologic responsiveness of the smooth muscle itself. We chose to examine drug responses in the trachealis muscle 1) because it represents a large, homogeneous sample of airway smooth muscle (Stephens, 1976) that would be suitable for further studies at a biochemical level and 2) because samples of trachealis muscle could be accurately matched between different breeds of dog. The latter aspect is of crucial importance for a comparative study, as previous work (G. A. Rinard, personal communication) has shown a large cephalocaudal gradient in the methacholine sensitivity of trachealis muscle. Little information is available concerning drug gradients in the distal bronchi and bronchioles, and it would be difficult to ensure that smaller airways isolated from BG dogs and greyhounds represented comparable tissue.

The BG dogs used in the present study showed the typical airway hyperresponsiveness (table 1) found in other BG dogs. These dogs, moreover, were not used in other studies so that changes in muscle responsiveness were not the result of repeated aerosol challenges. Similarly, none of the animals were sensitized intentionally by antigen exposure, so that differences between the groups presumably reflect changes associated with airway hyperresponsiveness rather than the process of antigen sensitization. One animal in each group was found to be naturally allergic to *Ascaris* antigen on aerosol challenge, but responses of tracheal muscle obtained from these animals fell within the mean ± 1.1 S.D. for their respective groups.

When matched preparations of trachealis muscle from BG and greyhound dogs were compared *in vitro*, those from BG dogs were either less sensitive (methacholine; table 2; fig. 2) or equally sensitive (histamine) than equivalent preparations from greyhounds. Similarly, the maximal contractile response, when normalized as amplitude of contraction/muscle weight was not significantly different in BG dogs and greyhounds. Therefore, our studies provide no evidence of an increased smooth muscle response as a basis for the rather marked airway hyperresponsiveness to methacholine and histamine observed *in vivo*. On the contrary, *in vitro* responsiveness to methacholine (but not histamine) showed a significant negative correlation with airway responsiveness *in vivo* (fig. 3). The correlation could represent an accidental juxtaposition of two independent variables, in two small and rather homogenous populations. However, the one greyhound that was relatively hyperresponsive to methacholine and histamine *in vivo* also supplied the least responsive of the greyhound tracheal preparations *in vitro*, suggesting something more than accidental juxtaposition. An inverse relationship between responsiveness *in vivo* and *in vitro* (if real), suggests an intrinsic adaptive mechanism, operative at the level

of the muscle cell, which acts to dampen the excessive responses initiated by mechanisms extrinsic to the muscle cell.

Studies using human airway smooth muscle obtained during thoracotomy (Vincenc *et al.*, 1983; Roberts *et al.*, 1984; Armour *et al.*, 1984a,b) have failed to demonstrate any relationship between responsiveness to methacholine or histamine *in vivo* (aerosol challenge) and *in vitro* (bronchial spirals or rings and lung parenchymal strips); however, most of the patients were in the older age group, few, if any, had symptomatic asthma and tissue sampling necessarily varied between subjects. Therefore, the possibility of a significant negative correlation between responses *in vivo* and *in vitro* should remain open until more work has been done with muscle samples from patients with reactive airway disease.

Previous studies (Chan *et al.*, 1985) have shown a blunted leucocyte cyclic nucleotide response to *beta* adrenergic stimulation in BG dogs and, in the present study, isoproterenol-induced relaxation required higher doses and had a lesser maximal effect (figs. 5 and 6) in BG preparations compared with equivalent preparations from greyhounds. Cholinergic agonists are known to produce a functional antagonism of isoproterenol (Buckner and Saini, 1975), and to reduce both the potency and efficacy of isoproterenol-induced relaxation in canine trachealis muscle (Russell, 1984; Torphy *et al.*, 1983, 1985). The differences in relaxation in our studies, therefore, may have resulted from the higher concentrations of methacholine needed to contract preparations from BG dogs, rather than a primary defect in the *beta* adrenergic responsiveness of BG smooth muscle. When responses are compared in BG (cervical) and greyhound (thoracic) preparations that were contracted by roughly similar concentrations of methacholine (figs. 5 and 6), their responses to isoproterenol are identical (table 3).

A cephalocaudal gradient for isoproterenol sensitivity paralleling that for methacholine sensitivity has been shown by Puckett *et al.* (1985) in greyhounds. In our studies, the relationship between the ED_{50} values for isoproterenol and methacholine is approximately linear (fig. 5) over more than 2 orders of magnitude (from BG thoracic trachealis to greyhound cervical trachealis) and the high concentrations of isoproterenol needed to relax BG muscle seem to be no more than an extension of the relationship as seen in greyhound trachealis muscle.

In summary, our studies provide no evidence that an abnormal sensitivity of airway smooth muscle to pharmacologic agonists contributes to the airway hyperresponsiveness characteristic of the BG dog, and suggest that its causes are extrinsic to the smooth muscle cells.

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References

ARMOUR, C. L., BLACK, J. L., BEREND, N. AND WOOLCOCK, A. J.: The relationship between bronchial hyperresponsiveness to methacholine and airway smooth muscle structure and reactivity. Respir. Physiol. **58:** 223–233, 1984a.

ARMOUR, C. L., LAZAR, N. M., SCHELLENBERG, R. R., TAYLOR, S. M., CHAN, N., HOGG, J. C. AND PARÉ, P. D.: A comparison of *in vivo* and *in vitro* human airway reactivity to histamine. Am. Rev. Respir. Dis. **129:** 907–910, 1984b.

ANTONISSEN, L. A., MITCHELL, R. W., KROEGER, E. A., KEPRON, W., TSE, K. S. AND STEPHENS, N. L.: Mechanical alterations of airway smooth muscle in a canine asthmatic model. J. Appl. Physiol. **46:** 681–687, 1979.

BUCKNER, C. K. AND SAINI, R. K.: On the use of functional antagonism to estimate dissociation constants for *beta* adrenergic receptor agonists in isolated guinea-pig trachea. J. Pharmacol. Exp. Ther. **194:** 565–574, 1975.

BUTLER, J. M., PETERS, J. E., HIRSHMAN, C. A., WHITE, C. R., MARGOLIN, L. B. AND HANIFIN, J. M.: Pruritic dermatitis in asthmatic basenji-greyhound dogs: A model for human atopic dermatitis. J. Am. Acad. Dermatol. **8:** 33–38, 1983.

CHAN, S. C., HANIFIN, J. M., HOLDEN, C. A., THOMPSON, W. J. AND HIRSHMAN, C. A.: Elevated leukocyte phosphodiesterase as a basis for depressed cyclic adenosine monophosphate responses in the basenji-greyhound dog model of asthma. J. Allergy Clin. Immunol. **76:** 148–158, 1985.

DOWNES, H. AND HIRSHMAN, C. A.: Importance of calcium in citric acid-induced airway constriction. J. Appl. Physiol. **55:** 1496–1500, 1983.

HIRSHMAN, C. A.: The Basenji-greyhound dog model of asthma. Chest **87:** 172S–178S, 1985.

HIRSHMAN, C. A. AND DOWNES, H.: Basenji-greyhound dog model of asthma: Influence of atropine on antigen-induced bronchoconstriction. J. Appl. Physiol. **50:** 761–765, 1981.

HIRSHMAN, C. A., DOWNES, H. AND VEITH, L.: Airway responses in offspring of dogs with and without airway hyperreactivity. J. Appl. Physiol. **56:** 1272–1277, 1984.

HIRSHMAN, C. A., MALLEY, A. AND DOWNES, H.: Basenji-greyhound dog model of asthma: Reactivity to *Ascaris suum*, citric acid, and methacholine. J. Appl. Physiol. **49:** 953–957, 1980.

HIRSHMAN, C. A., PETERS, J., BUTLER, J., HANIFIN, J. M. AND DOWNES, H.: Role of mediators in allergic and nonallergic asthma in dogs with hyperreactive airways. J. Appl. Physiol. **54:** 1108–1114, 1983a.

HIRSHMAN, C. A., PETERS, J., DOWNES, H., LEON, D., LYNN, R. K., BUTLER, J. AND HANIFIN, J. M.: Citric acid airway constriction in dogs with hyperreactive airways. J. Appl. Physiol. **54:** 1101–1107, 1983b.

KAUKEL, E., BEIER, W., FEDDERSEN, C., SCHRUM, C. AND KOPPERMANN, G.: Mechanical and biochemical alterations in guinea-pig tracheae caused by sensitization. Respiration **45:** 255–261, 1984.

MORCILLO, E. J., PERPIÑÁ, M. AND ESPLUGUES, J.: Hyperresponsiveness to autacoids and autonomic drugs in lung parenchymal strips from sensitized guinea pigs. Am. Rev. Respir. Dis. **129:** 948–951, 1984.

PUCKETT, A. M., RINARD, G. A., JENSEN, A. D., MINNEMAN, K. L., TORPHY, T. J. AND MAYER, S. E.: Variation in β-adrenergic-cholinergic sensitivity balance and imbalance in the trachealis smooth muscle of greyhound dogs. Fed. Proc. **44:** 455, 1985.

ROBERTS, J. A., RAEBURN, D., RODGER, I. W. AND THOMSON, N. C.: Comparison of *in vivo* airway responsiveness and *in vitro* smooth muscle sensitivity to methacholine in man. Thorax **39:** 837–843, 1984.

RUBINFELD, A. R., RINARD, G. A. AND MAYER, S. E.: Responsiveness of isolated tracheal smooth muscle in a canine model of asthma. Lung **160:** 99–107, 1982.

RUSSELL, J. A.: Differential inhibitory effect of isoproterenol on contractions of canine airways. J. Appl. Physiol. **57:** 801–807, 1984.

SNAPPER, J. R., DRAZEN, J. M., LORING, S. H., SCHNEIDER, W. AND INGRAM, R. H.: Distribution of pulmonary responsiveness to aerosol histamine in dogs. J. App. Physiol. **44:** 738–742, 1978.

SOUHRADA, J. F.: Changes of airway smooth muscle in experimental asthma. Respir. Physiol. **32:** 79–90, 1978.

SOUHRADA, M., KLEIN, J. J., BEREND, N. AND SOUHRADA, J. F.: Topographical differences in the physiological response of canine airway smooth muscle. Respir. Physiol. **52:** 245–258, 1983.

STEPHENS, N. L.: Airway smooth muscle. *In* Lung Cells in Disease, ed. by A. Bouhuys, pp. 113–138, Elsevier/North-Holland, Amsterdam, 1976.

TAYLOR, S. M., DOWNES, H., HIRSHMAN, C. A., PETERS, J. E. AND LEON, D.: Pulmonary uptake of mannitol as an index of changes in lung epithelial permeability. J. Appl. Physiol. **55:** 614–618, 1983.

TORPHY, T. J., RINARD, G. A., RIETOW, M. G. AND MAYER, S. E.: Functional antagonism in canine tracheal smooth muscle: Inhibition by methacholine of the mechanical and biochemical responses to isoproterenol. J. Pharmacol. Exp. Ther. **227:** 694–699, 1983.

TORPHY, T. J., ZHENG, C., PETERSON, S. M., FISCUS, R. R., RINARD, G. A. AND MAYER, S. E.: Inhibitory effect of methacholine on drug-induced relaxation, cyclic AMP accumulation and cyclic AMP-dependent protein kinase activation in canine tracheal smooth muscle. J. Pharmacol. Exp. Ther. **233:** 409–417, 1985.

VINCENC, K. S., BLACK, J. L., YAN, K., ARMOUR, C. L., DONNELLY, P. D. AND WOOLCOCK, A. J.: Comparison of *in vivo* and *in vitro* responses to histamine in human airways. Am. Rev. Respir. Dis. **128:** 875–879, 1983.

VON NEERGAARD, K. AND WIRZ, K.: Die Messung der Strömungswiderstände in den Atemwegen des Menschen, insbesondere bei Asthma und Emphysem. Z. Klin. Med. **105:** 51–82, 1927.

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