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The antagonism of histamine-induced tracheal and bronchial muscle contraction by diphenhydramine: effect of maturation

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1 Airway reactivity in spontaneously breathing unanaesthetized female guinea-pigs was significantly reduced as a consequence of maturation. Threshold doses to histamine (% w/v base) were 0.08% ± .01 and 0.27% ± .04 in immature (110g ± 2; 1 week old) and mature (837 g ± 29; 4 months old) animals, respectively.

2 The potency of 2-(2-thiazolyl) ethylamine in tracheal tissues from mature animals was significantly less than that in tissues from immature animals (5.06 ± .03 vs 5.26 ± .07). There was no change in the potency of this agonist in bronchial tissues (5.0 ± .09 vs 4.9 ± .13).

3 Diphenhydramine reduced tissue contractility in tracheal (30% at 0.1 μM; 50% at 3 μM) but not bronchial tissues. The antihistamine in concentrations ranging from 0.1 μM to 3 μM reduced the potency of histamine 2 to 50 fold in both tissues.

4 Schild plots were linear but slopes were significantly less than unity. pA_2 values ± 95% fiducial limits derived from data from tracheal tissues of immature and mature animals using constrained Schild plots (unit slope) were 7.7 (7.6–7.7) and 7.1 (7.0–7.2), respectively ($P < 0.05$). pA_2 values for diphenhydramine in bronchial tissues using constrained Schild plots were 7.8 (7.7–7.9) and 7.5 (7.3–7.5), respectively ($P < 0.05$).

5 The data emphasize the unique nature of tracheal and bronchial tissues. We conclude that, with maturation, the characteristics of the histamine receptor change. We suggest that the remission of asthma which frequently occurs at puberty may be related to alteration in the properties of membrane receptors.

Introduction

Airway smooth muscle contraction to histamine has been shown to result from the binding of the agonist to a membrane receptor. The chain of subsequent events include altered calcium sequestration and transport (Somylo & Somylo, 1968; Hurwitz & Suria, 1971), altered phospholipid metabolism (Orehek *et al.*, 1974; Brink *et al.*, 1981a; Villalobos-Molina & Garcia-Sainz, 1983) and the elevation of intracellular cyclic nucleotide levels (Duncan *et al.*, 1980; Brink *et al.*, 1981a). Recently, it has been reported that the potency of agonists is reduced during maturation both *in vivo* and *in vitro* (Brink *et al.*, 1980; Duncan *et al.*, 1982; Duncan & Douglas, 1983; Douglas *et al.*, 1982). Altered potency may be due to changes in receptor affinity, efficacy or other factors contributing to the biological response. To determine whether the histamine receptor changed as a consequence of maturation, a study was initiated which examined the effects of the H_1-receptor antagonist, diphenhydramine and the H_1-receptor agonist 2-(2-thiazolyl) ethylamine on respiratory tissues from immature and mature guinea-pigs. Preliminary results of these studies have been recently presented (Duncan *et al.*, 1983; Mukhopadhyay *et al.*, 1983).

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Methods

Female albino guinea-pigs (Hartley strain, Camm Research Institute Inc., Wayne, N.J.) were used in this study. The average weights of immature and mature animals were 110g ± 2 (< 1 week old) and 837 g ± 29 (> 4 months old), respectively.

In vivo *experiments*

Each animal was placed in a body plethysmograph from which tidal volume was recorded (Douglas *et al.*, 1972). Thirty minutes after the guinea-pig was placed in the plethysmograph, it was exposed for 30 s to an aerosol of saline or histamine produced with a Petersen-Rooth generator. The aerodynamic particle size from the generator was 1–5 microns (Rooth, 1949). Each challenge with histamine was preceded by a control challenge of 0.9% w/v saline. The initial histamine concentration was 0.01% w/v histamine for immature animals and 0.05% w/v histamine for mature animals. Subsequent doses were selected on the basis of the responses obtained to the initial concentrations. An interval of approximately 30 min was allowed for recovery between challenges. The threshold dose was defined as the concentration of histamine in the aerosol generator which produced a 10% reduction in tidal volume, provided that the concentrations of histamine immediately higher and lower elicited positive and negative reactions, respectively (Popa *et al.*, 1973). Tissues from these animals were used for the *in vitro* studies described below so that the relationship between the threshold dose to histamine and the dissociation constant (pK_b) for diphenhydramine could be examined (see below). *In vivo* airway reactivity to histamine was determined one to five days before tissues were removed for use *in vitro*.

In vitro *experiments*

Tissue preparation Guinea-pigs of known age were killed by exsanguination; lungs and tracheae were dissected intact and placed in Tyrode solution. The composition of Tyrode solution was as follows (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4, 0.4 and glucose 5.5, at pH 7.4. The tracheae and bronchi were dissected free from adhering extraneous tissues, cut spirally, mounted in tissue baths and then equilibrated for 90 min under initial loads of 10 g and 2 g, respectively, at 37 °C in Tyrode solution gassed with 5% CO_2 in O_2 (Brink *et al.*, 1980). During the equilibration period, the bath fluid was exchanged for fresh Tyrode solution at 15 min intervals and the preparations were allowed to relax passively. At the end of the equilibration period, the length of each preparation was measured. Forces developed were monitored isometrically with Statham strain gauges (model UC3) and the output signals were recorded by Honeywell (Electronik 19) dual channel or Heath single channel pen recorders.

Concentration-effect curves to histamine At the end of the equilibration period, all preparations were contracted with a maximally-effective concentration of histamine (50 μM) i.e. tissues were primed (Brink *et al.*, 1981b). In preliminary experiments, two concentration-effect curves were generated in each tissue. One was achieved using cumulative drug additions while in the other responses to single concentrations of agonist were assessed with complete washout interposed. The order in which the two protocols were used for a given tissue was chosen randomly. To ascertain whether histamine-induced tone was sustained for the duration of a cumulative concentration-effect curve, a concentration of histamine was added which induced an increase in tension of approximately 50% of maximal. The induced tone was monitored for 60 min. The reproducibility of cumulative concentration-effect curves was determined by generating three curves separated by two 30 min incubations in Tyrode solution i.e. sham incubations. Since both single addition and cumulative addition protocols gave identical results (see results) and the latter protocol was much quicker, all subsequent experiments were carried out using the cumulative drug addition protocol (Van Rossum, 1963). In addition, cumulative concentration-effect curves to 2-(2-thiazolyl) ethylamine were generated in tracheal and bronchial tissues from immature ($n = 6$) and mature ($n = 4$) guinea-pigs.

Antagonism of histamine response by diphenhydramine An initial cumulative concentration-effect curve to histamine was established. The tissue was washed, allowed to return passively to the original basal tone, then incubated for 30 min with diphenhydramine (0.1 μM–3 μM). A new cumulative concentration-effect curve to histamine was established in the presence of the antihistamine and the preparation again washed to re-establish basal tone. The tissue was then incubated for 30 min with a higher concentration of the antagonist and a new histamine concentration-effect curve generated in the presence of this concentration of antihistamine. In each experiment, tissues were treated with two concentrations of diphenhydramine. At the end of the antagonist assay, tissues were washed to baseline. They were then contracted with a maximally-effective concentration of carbachol (10 μM) and relaxed with a maximally-effective concentration of aminophylline (2 mM) in order to determine the effects of the antagonist treatment on maximal contraction/relaxation of the tissues. Finally, tissues were removed from the tissue baths, blotted dry and weighed to allow a calculation of cross-sectional area (Brink *et al.*, 1980).

Calculation of results

In experiments in which the two methods for generat-

ing concentration-effect curves were used, the data were normalized with respect to the response to 50 μM histamine in the single random addition protocol. When repeated cumulative concentration-effect curves were used, the data were normalized relative to the maximal response in the initial concentration-effect curve. The 'priming' concentration was not used for any of the calculations. EC_{50} values to histamine or 2-(2-thiazolyl) ethylamine were interpolated from concentration-effect curves and were transposed into logarithms (pD_2 values). The contractility of the agonists was the ratio: maximal induced force/cross-sectional area (Brink *et al.*, 1980). The affinity of diphenhydramine was determined using standard Schild plots (Arunlakshana & Schild, 1959) from which pA_2 and pA_{10} values were determined. In addition, pA_2 values were determined using constrained Schild plots (Tallarida & Murray, 1981). These results were compared with calculated data using the formula, $pK_b = -\log(\text{antagonist concentration})/(\text{dose ratio} - 1)$ (Furchgott, 1967). Values shown are the means ± s.e.mean or the means ± fiducial confidence limits. Statistical comparisons were made using Students *t* tests for paired or unpaired variates as appropriate.

Drugs and their sources

Diphenhydramine hydrochloride, histamine dihydrochloride, carbamylcholine chloride and aminophylline were purchased from Sigma Chemical Company, St Louis, Mo. 2-(2-Thiazolyl) ethylamine was a gift from Smith, Kline & French Laboratories, Philadelphia, PA, U.S.A.

Results

In vivo *responses to histamine*

In this cross sectional study of airway reactivity, the threshold dose to histamine in immature guinea-pigs (110 g ± 2; approximately 1 week old) was

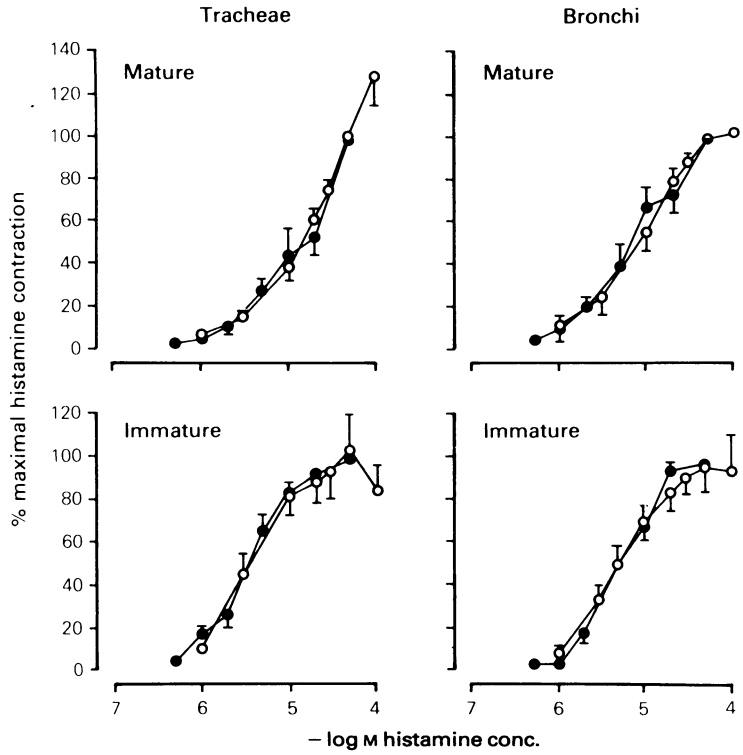

Figure 1 Effect of histamine on isolated tracheal and bronchial tissues from immature and mature female guinea-pigs. Concentration-effect curves to histamine were generated using single addition (●) or cumulative addition (○) protocols (see methods). Each point represents the mean of 5–8 experiments with s.e.mean shown by vertical lines. The response to 50 μM histamine was considered to be maximal i.e. 100%. Slopes of the concentration-effect curves and maximal induced tensions were not significantly different using the two protocols.

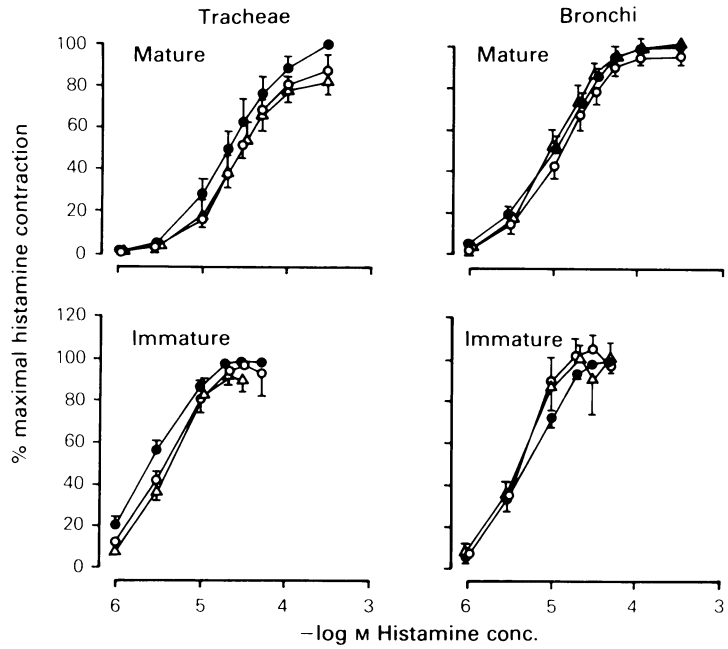

Figure 2 Effect of vehicle incubation on cumulative concentration-effect curves of respiratory tissues from immature and mature female guinea-pigs to histamine. All panels represent control (●) and two successive (○; △) cumulative curves, each obtained after a 30 min incubation with vehicle. No significant changes in pD_2 values or maximum tensions were observed. Every point is the mean of 6–8 experiments; vertical lines indicate s.e.mean.

0.08% ± .01 ($n = 20$). These animals were significantly more sensitive to histamine ($P < 0.05$) than mature guinea-pigs (837 g ± 29; approximately 4 months old; $n = 14$) in which the mean threshold dose was 0.27% ± .04. The threshold dose for each animal was plotted against the ED_{50} to histamine or the pK_b for diphenhydramine for each isolated tissue from that animal. There was no correlation between *in vivo* histamine threshold and histamine potency *in vitro* or diphenydramine affinity *in vitro* for the two age groups (data not shown).

Histamine concentration-effect curves

Cumulative concentration-effect curves to histamine take 20 min and 40 min to generate in tissues from immature and mature animals, respectively. The slower rate of contraction and a slower recovery to baseline with washing contributed equally to the longer time taken to construct cumulative concentration-effect curves to histamine in tissues from mature animals. When tissues were contracted with an EC_{50} concentration of histamine, the induced tone was stable for at least 60 min, declining by no more than 15% (data not shown). These results suggested that the use of cumulative concentration-effect curves for this study might be possible provided that these curves were identical to concentration-effect curves generated in the same tissue using the single addition protocol. In the tissues examined, the two protocols did yield identical concentration-effect curves (Figure 1). Histamine was most potent in tracheal tissues from immature animals and potency was reduced in tissues from mature animals. These results extend previously published results from this laboratory (Brink *et al.*, 1980). Similarly, the potency of 2-(2-thiazolyl) ethylamine, the H_1 specific agonist, was greatest in tissues from immature animals and was reduced as a consequence of maturation.

Repeated cumulative concentration-effect curves, separated by a 30 min incubation period in Tyrode solution, were reproducible (Figure 2). Tissues treated with diphenhydramine (100 nM–3 μM) showed a decreased sensitivity to histamine which was dependent upon the concentration of antagonist used (Figure 3) reflecting the antagonist activity of the compound. Preliminary experiments showed that the contact time with the antihistamine was adequate

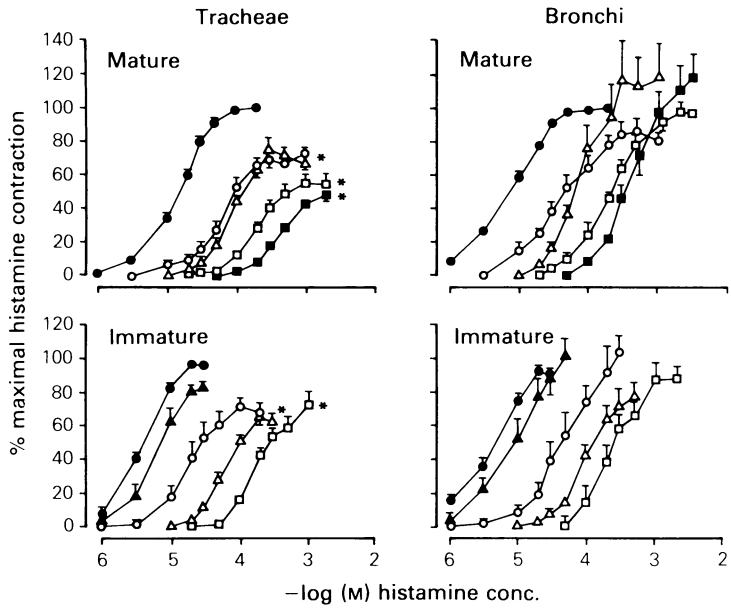

Figure 3 Competitive antagonism of histamine receptors by diphenhydramine in tracheal and bronchial smooth muscle for immature and mature female guinea-pigs. Each panel shows the control concentration-effect curve to histamine (●) and the parallel shift to the right in the presence of diphenhydramine at 10 nM (▲), 100 nM (○), 300 nM (△), 1 μM (□) and 3 μM (■). Each point represents the mean with s.e.mean shown by vertical lines of 5–12 experiments. Maximum contractility in the control histamine concentration-effect curve was 100%. * indicates significant differences ($P < 0.05$) in the maximum tension induced by histamine in diphenhydramine-treated tissues compared to control.

to achieve equilibrium in all the tissues studied. In tracheal but not bronchial tissues, the antihistamine also significantly decreased the contractility of histamine at the three highest concentrations tested. Schild plots to evaluate the potency of the antagonist gave regression lines with significant correlation coefficients (0.88–0.96) but with slopes significantly less than unity (Table 1, Figure 4; see Discussion). Using weighted regression analysis (i.e. lines with a slope of −1) pA_2 values (Table 1) were identical to the mean calculated values. If antagonist affinity was calculated using concentration-effect curve data before and after treatment with the lowest concentration of diphenhydramine (experiments where there was no significant change in tissue contractility), the result was unchanged. That is, diphenhydramine was significantly less potent in tracheal tissues from mature animals than in tracheal tissues from immature animals (Figure 4; Table 1). The antagonist was also slightly more potent in bronchial tissues from immature animals.

Carbachol contracted all of the tissues tested to a greater degree than histamine. The rank order for tension development using carbachol as the agonist was immature trachea > mature trachea > immature bronchus = mature bronchus. Tensions ($g\,mm^{-2}$), in vehicle incubated tissues, i.e. not treated with antihistamine, were 1.64 ± .23, 1.10 ± .14, 0.28 ± .03 and 0.31 ± .05 for these tissues, respectively. Treatment with diphenhydramine did not measurably affect the response to carbachol or aminophylline when compared to control data.

Discussion

In a previous study we noted that airway sensitivity to histamine decreased with age, especially in tracheal tissues (Brink *et al.*, 1980). Using cumulative concentration-effect curves, we confirm and extend these observations. In experiments designed to elucidate the site at which this change occurs, we have defined the properties of the H_1-receptor in these tissues using the H_1 agonist 2-(2-thiazolyl) ethylamine and the H_1 antagonist, diphenhydramine. We show that the potencies of 2-(2-thiazolyl) ethylamine and histamine were reduced in tracheal tissues as a consequence of maturation. The results with 2-(2-thiazolyl) ethylamine show that matura-

Table 1 Histamine receptor characteristics and maturation

Tissue	Age	2-(2-Thiazolyl) ethylamine (pD_2, n)	Diphenhydramine† (pA_2, n)
Tracheae	Immature	5.26 ± 0.07 (6)*	7.67 (7.60–7.73; 40)* 7.94 (7.84–8.04)*
	Mature	5.06 ± 0.03 (4)	7.10 (7.00–7.20; 27) 7.34 (7.32–7.35)
Bronchi	Immature	5.00 ± 0.09 (5)	7.82 (7.71–7.92; 30)* 8.05 (7.84–8.25)
	Mature	4.90 ± 0.13 (4)	7.46 (7.36–7.56; 22) 7.88 (7.53–8.22)

† The upper pA_2 values are derived from constrained Schild plots. The lower values are from least squares fitted lines (Figure 4). Number of experiments and 95% confidence limis are shown.
* Values significantly different from those in tissues from mature animals ($P < 0.05$).
Values are mean ± s.e.mean.

tional changes at the H_1 receptor are responsible for the decreased potency of histamine.

To probe this possibility further, we examined the effects of maturation on receptor affinity using the H_1 antagonist, diphenhydramine. This agent decreased the potency of histamine in tracheal and bronchial tissues and reduced tissue contractility to histamine only in tracheal preparations. pA_2 values were identical when determined by calculation or constrained Schild analysis (Table 1). This analysis was necessary because the slopes of normal Schild plots were significantly different from unity i.e. the slope ± 95% fiducial limit was < 1 (Figure 4). The reason for the deviation from unity is unclear. Uptake and metabolism of histamine seem unlikely contributing factors since contractile responses to histamine were sustained and did not fade with time (see results). Neither can the deviation of the Schild plots from unity be due to reduced tissue contractility because bronchial tissue contraction was unaffected by diphenhydramine yet data analysis still gave Schild plots less than one. The pA_2 values reported here (Table 1) and the difference between pA_2 and pA_{10} i.e. 1.19–1.30 have been seen with diphenhydramine and other antihistamines in other tissues. Although possible, the interaction of histamine with other binding sites (e.g. cholinoceptor, H_2) is an unlikely explanation for these maturational changes because the potency of the specific H_1 receptor agonist, 2-(2-thiazolyl) ethylamine, was also decreased.

Reported pA_2 values for this antagonist in the jejunum and ileum of the guinea-pig are 7.50 and 8.14, respectively (Marshall, 1955; Van Rossum *et al.*, 1958). Our results with respiratory tissues (Figure 4) are virtually the same. However, there are significant differences in the pA_2 values of diphenhydramine between tracheal and bronchial tissues from immature and mature guinea-pigs (Table 1). High values for the pA_2-pA_{10} for diphenhydramine have been reported in other tissues, for example, guinea-pig ileum (Marshall, 1955). Marshall (1955) proposed that antihistamines with pA_2-pA_{10} values ranging from 0.8 to 1.3 could be considered as competitive antagonists. The same author examined several competitive and non-competitive antihistamines and concluded that the aberrant characteristics of diphenhydramine might be associated with its anticholinoceptor activity. No anticholinoceptor activity of diphenhydramine, in the concentration range 0.1 μM to 3 μM, was demonstrable against carbachol. If anticholinoceptor activity had been evident, interpretation of the result would have been complicated by other data which show that functional responses to histamine and carbachol decrease in quantitatively comparable manner with maturation (Douglas *et al.*, 1982). Antagonist data show that the altered affinity of diphenhydramine is specifically related to altered H_1 receptor properties. The results agree with other observations which show that the changes in the potency of agonists *in vitro* during maturation are due to altered receptor characteristics. The results also point to differences between tissues from different sites within the same organ.

Most β-adrenoceptor blocking agents were developed using tracheal and cardiac smooth muscle preparations. The selectivity of these agents was determined on the basis of pA_2 values in these tissues

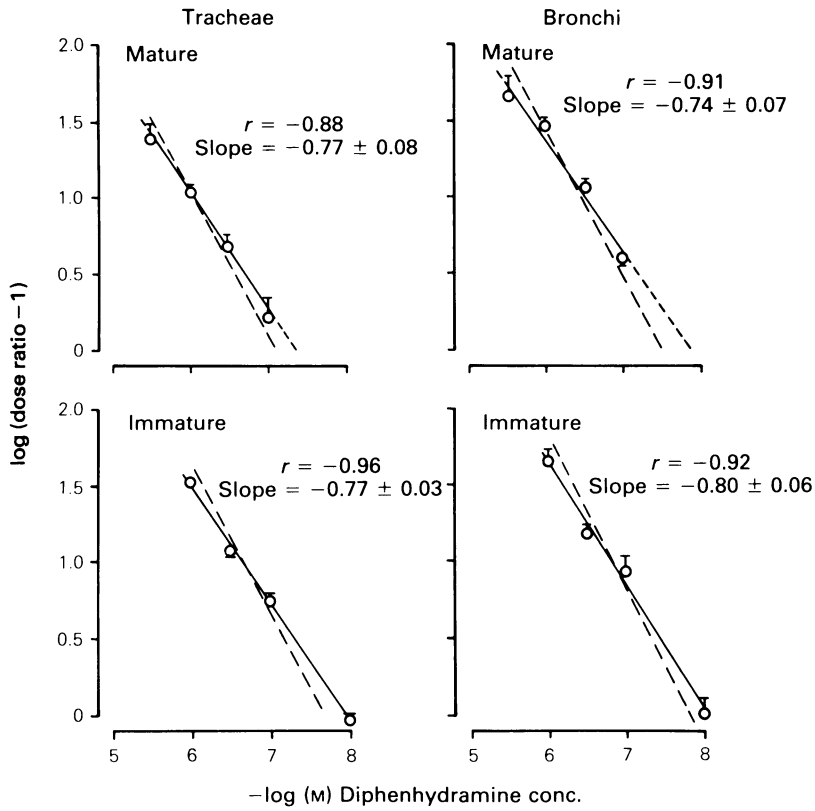

Figure 4 pA_2 values of diphenhydramine in respiratory tissues from immature and mature female guinea-pigs. Unweighted regression lines (continuous) for the antagonism of histamine by diphenhydramine in tracheal and bronchial smooth muscles are shown. Each data point is the mean of 5–12 observations; vertical lines represent s.e.mean. Inserts in each panel show the correlation coefficient (r) and the mean ± s.e.mean of the slope. Constrained Schild plots (unit slope, dashed lines) are also shown for the same data. pA_2 values were determined from intercepts of the weighted regression lines through the data points with the abscissae. Statistically significant differences were observed for pA_2 values ($P < 0.05$) between tracheal tissues from mature animals and tracheal and bronchial tissues from immature animals and also between bronchial tissues from mature animals (see also Table 1).

(O'Donnell *et al.*, 1980). The results in this paper show that the pA_2 value of an antagonist in different tissues from the same organ may differ. Similarly, Mukhopadhyay *et al.* (1982) reported differences in the activity of β-adrenoceptor agonists between tracheal and parenchymal tissues. Therefore, use of tracheal smooth muscle alone may not yield accurate data for the determination of the bronchoselectivity of an agent. The selection of tissues for these assays should take into account potential site(s) of action and even the age of animals used.

Airway sensitivity to histamine was significantly greater in immature guinea-pigs and decreased as a consequence of maturation. The results of this cross-sectional study have been verified in a study in which airway reactivity was followed throughout the lifetime of an animal i.e. a longitudinal study (Duncan *et al.*, 1983). In order to relate antihistaminic activity *in vitro* with histamine sensitivity *in vivo*, the tissues used *in vitro* were taken from animals of known airway reactivity. There was no correlation between airway reactivity *in vivo* and the potency of histamine or diphenhydramine *in vitro*. In a previous study, we reported that *in vitro* histamine sensitivity was not correlated with *in vivo* histamine threshold. The inability to demonstrate such a correlation probably reflects the fact that the bronchoconstrictor threshold *in vivo* represents a summation of many stimuli interacting at smooth muscle and modulating its response e.g. neuronal inputs, circulating humors and hormones.

The reduced potency of bronchoconstrictors which

occurs as a consequence of maturation provides one explanation for the high remission rate of childhood bronchial asthma during adolescence (Rackemann & Edwards, 1952; Smith, 1971; McNicol & Williams, 1973; Broder *et al.*, 1974). It is unclear from these studies precisely when these changes occur and whether or not they are the consequence of vigorous therapy. A greater sensitivity of airway smooth muscle in children as compared to adults has been reported for air pollutants such as SO_2 (Schlenker & Jaeger, 1980). The immunological release of mediators from lung tissue is also greater in tissues from immature animals (Fleisch *et al.*, 1978). If both processes show reduced activity during maturation, then a lower incidence of allergic asthma might be expected. Data from our animal model show that airway reactivity *in vivo* changes at or around puberty (Duncan *et al.*, 1983) suggesting that the hormones of puberty can significantly affect the properties of drug receptors in the lung.

In some patients with hyperthyroid disease, asthma is a complicating factor. Successful treatment of the hyperthyroid state results in a loss of asthmatic symptoms and about a five fold decrease in airway reactivity (Cockcroft *et al.*, 1978). The changes in receptor affinity which occur as a consequence of maturation could therefore account for reduced airway reactivity. Whether the altered biological response is due to increased numbers of spare receptors, changes in receptor density and/or changes in the proportion of high and low affinity sites of the receptor is the subject of a current investigation. The results with diphenhydramine, histamine and 2-(2-thiazolyl) ethylamine do, however, show that the properties of the H_1-receptor are subject to maturational changes that can significantly affect the consequences of drug/receptor interaction. We expect that our animal model will continue to provide additional insights into these mechanisms.

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