Br. J. Pharmac. (1982), **75**, 531–537

CHANGES IN THE RESPONSE OF GUINEA-PIG AIRWAYS *in vivo* AND *in vitro* TO CIMETIDINE AND PROPRANOLOL DURING DEVELOPMENT

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1 Airway responses were examined in isolated tissues and in whole animal preparations of female albino guinea-pigs of known age.

2 Tone induced with acetylcholine in tracheal and bronchial tissues from young and old female guinea-pigs was not reduced by dimaprit or 4-methyl histamine even in tissues pretreated with mepyramine maleate.

3 Antagonism of H_2-receptors with cimetidine did not affect the potency or efficacy of histamine in tracheal tissues from animals of either age group.

4 After cimetidine treatment the potency of histamine was increased in bronchial tissues from old but not young animals. The sensitizing effect was still demonstrable in tissues incubated with indomethacin.

5 *In vivo* airway sensitivity to threshold concentrations of histamine in animals from either age group was unaffected by cimetidine treatment.

6 Propranolol enhanced airway responses to histamine aerosols in young but not old guinea-pigs.

7 Cimetidine was without effect on histamine sensitivity in young guinea-pigs after propranolol treatment but significantly reduced airway sensitivity to histamine in old guinea-pigs.

8 Our data show that (a) H_2-receptors are of no physiological significance for airway responses to histamine *in vitro* or *in vivo* and (b) during development the modulating actions of catecholamines upon airway responses are significantly reduced.

Introduction

Histamine can induce airway muscle contraction or relaxation *in vitro*. These divergent effects are believed to be mediated by two distinct binding sites for histamine, namely H_1- and H_2-receptors (Eyre, 1977). In airway muscle preparations from guinea-pigs the presence of both receptor subtypes has been suggested (Okpako, Chand & Eyre, 1978; Drazen, Schneider & Venugopalan, 1979), but H_2-receptor stimulation is of questionable significance (Maconochie, Woodings & Richards, 1979). For example, Duncan, Brink, Adolphson & Douglas (1980) were able to demonstrate an elevation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) which was associated with H_2-receptor stimulation but not with *in vitro* muscle relaxation. In contrast, other authors (Okpako *et al.*, 1978; Drazen *et al.*, 1979; Yen & Kreutner, 1979) have suggested that H_2-receptor stimulation in this species attenuates histamine-induced muscle contraction. Since histamine receptors may be altered qualitatively and/or quantitatively during aging (Schneider, Drazen, Snapper, Loring & Ingram, 1978), we have examined the physiological role of H_2-receptors in airway preparations from young and old guinea-pigs.

Methods

Female albino guinea-pigs (Hartley strain, Camm Research Inc., Wayne, N.J.) were used in this study. Young female animals (1 week old) weighed 91.8 g ± 3.2 when killed; old female animals (> 90 days old) weighed 748.2 g ± 30.7.

Guinea-pig airways in vitro

Histamine Tracheal and bronchial spirals from female guinea-pigs were equilibrated in Tyrode solution at 37°C (gassed with 5% CO_2 in O_2) under an initial load of 10 g and 2–3 g, respectively (Brink, Duncan, Midzenski & Douglas, 1980a). The high initial loads ensured that, after the 1.5 h equilibration period, the resting tension was optimal and responses to agonists were maximal and reproducible. Changes

0007-1188/82/030531-07 $01.00

in force were measured isometrically with Statham strain gauges (model UC3) and were displayed on Honeywell two channel pen recorders (Electronik 19). Cross sectional area was determined as a ratio of the length of the preparation (measured at the end of the equilibration period) to the wet weight of the preparation (determined after the experiment; Brink *et al.*, 1980a). Cross sectional area is necessary for the determination of tension. The composition of the Tyrode solution was (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 11.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5, pH 7.4. Concentrations of histamine, in a volume less than 0.5 ml, beginning with the highest histamine dose (final bath concentration, 50 μM) were added to the organ bath. Subsequent concentrations were added in random order to determine a concentration-effect curve. When the response to agonist reached a plateau, the bath fluid was exchanged for fresh Tyrode solution. An interval of approximately 15 min was necessary to allow the preparation to return passively to its resting tension. A new concentration of agonist was then added to the bath. Concentration-effect curves were re-examined after preparations were treated with Tyrode solution (Brink, Duncan & Douglas, 1981) or with cimetidine (50 μM; Duncan *et al.*, 1980). The treatment period was always 30 min and the drug was washed out before a new concentration-effect curve was generated. EC_{50} values were interpolated by eye from hand-fitted histamine concentration-effect curves (see Results); pD_2 values were defined as the negative logarithm of the molar EC_{50} value (the concentration of agonist required to induce a tension which was 50% of maximum). pD_2 values are a measure of the tissue sensitivity to an agonist. The responsiveness of tissues to histamine was determined as previously described (Brink *et al.*, 1980a).

Dimaprit and 4-methyl histamine The physiological activities of H_2-agonists were examined in tracheal and bronchial tissues taken from the same animal. The agonists were tested in tissues from both young and old animals. Preparations were equilibrated as above. Cumulative concentration-effect curves to the relaxant agonists were generated after a tension was induced in the preparations with acetylcholine. We tested each preparation with acetylcholine in order to determine the concentration that was maximal but not supramaximal (20 to 50 μM). Induced tensions ranged from 20% to 100% of maximal. When the induced tension reached a plateau, the relaxant agonist was added to the bath as previously described (Foster, 1967; Douglas, Ridgway & Brink, 1977; Brink *et al.*, 1980a). The effects of dimaprit and 4-methyl histamine were also examined in preparations treated with mepyramine maleate (20 μM) for 30 min followed by washout, exposed to mepyramine maleate (20 μM) continuously from the beginning of the experiment or treated with mepyramine maleate (20 μM) 5 min before each addition of acetylcholine. Tissues were tested with histamine to demonstrate complete blockade of H_1-receptors.

Guinea-pig airways in vivo

Determination of threshold dose to histamine Each animal was placed in a body plethysmograph from which tidal volume, air flow rate and respiratory frequency were recorded (Popa, Douglas & Bouhuys, 1973). One half hour after the guinea-pig was placed in the plethysmograph, it was exposed for 30 s to an aerosol of histamine, produced with a Petersen-Rooth generator. Each challenge with histamine was preceded by a control challenge of 0.9% w/v NaCl solution (saline). Doses of histamine (usually in increments of 0.025% w/v histamine base) were administered in random order and were selected on the basis of responses obtained. A minimum of 30 min was allowed between successive challenges for tidal volume, air flow rate and respiratory frequency to return to control levels (Douglas, Dennis, Ridgway & Bouhuys, 1972).

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). Histamine threshold doses were determined in young and old guinea-pigs both before and after cimetidine (30 mg/kg, i.p., allowing 2 h for absorption). We also examined the effects of cimetidine in animals that had been pretreated with propranolol hydrochloride. We determined the threshold dose to histamine before, and 1 h after, propranolol treatment (10 mg/kg, i.p. as the hydrochloride). The animals were then given cimetidine (30 mg/kg, i.p.) and 1 h later another propranolol injection (10 mg/kg, i.p.). Threshold doses to histamine were re-examined 1 h after this propranolol injection.

Calculation of results

Data were compared by Student's *t* test for paired or unpaired variates as appropriate. Values presented are the means ± s.e.mean.

Materials

The drugs used, and their sources, were: histamine dihydrochloride, acetylcholine chloride (Sigma Chemical Company, St. Louis, Mo.); cimetidine, 4-methyl histamine dihydrochloride, dimaprit dihydrochloride (Smith, Kline and French Laboratories,

Philadelphia, Pa.); mepyramine maleate (R.W. Greeff & Co., New York, N.Y.); (±)-propranolol hydrochloride (Ayerst Laboratories, New York, N.Y.).

Results

Guinea-pig airways in vitro

The sensitivity and responsiveness of the bronchial preparations to histamine were the same as we reported previously (Table 1; Brink *et al.*, 1980a). Incubation of preparations with Tyrode solution did not alter tissue sensitivity or responsiveness to histamine (Figure 1). Left bronchial preparations from young guinea-pigs were not affected by cimetidine treatment (Figure 1a, Table 1) while left bronchial tissues from old guinea-pigs became sensitized to the agonist (Figure 1b, Table 1). Bronchial preparations from young and old animals were equally sensitive to histamine. Responsiveness in bronchial preparations from both young and old animals was not significantly changed after 'incubation' in Tyrode solution. Cimetidine treatment significantly increased the responsiveness of bronchial tissues from old animals (Table 1). Pretreatment of two bronchial spirals from old animals with indomethacin (17 μM) resulted in enhanced responsiveness to histamine without a change in tissue sensitivity (Brink *et al.*, 1980a). Cimetidine treatment in the presence of indomethacin caused a significant sensitization of these preparations to histamine and the magnitude of the sensitization was the same as that in preparations not treated with the anti-inflammatory drug (pD_2 value 6.06 vs. 5.49; Table 1). Neither dimaprit nor 4-methyl histamine induced bronchial relaxation in tissues from young or old animals even after treatment with mepyramine maleate (Figure 2). Similarly, dimaprit and 4-methyl histamine were physiologically inactive in old bronchial preparations pretreated with antihistamine and partially contracted with acetylcholine (Figure 3).

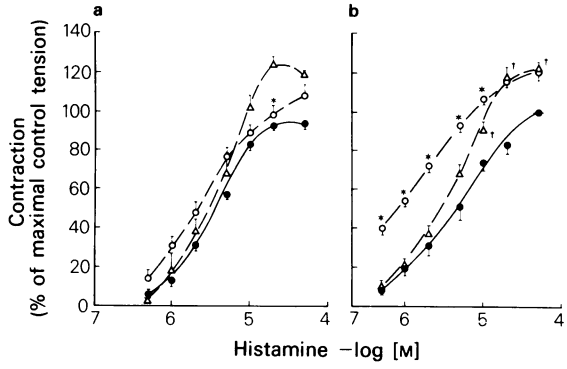

Figure 1 The effect of cimetidine in left bronchial preparations from young and old female guinea-pigs. Bronchial tissues were prepared as described in methods: (a) Concentration-effect curves to histamine before (●; $n = 9$) and after incubation with Tyrode solution (△; $n = 3$) or cimetidine (○, 50 μM; $n = 6$) in bronchial tissues from young guinea-pigs. (b) Identical experiments in tissues from old guinea-pigs; control (●; $n = 10$); Tyrode solution (△, $n = 4$); cimetidine (○, 50 μM; $n = 4$). The effect of cimetidine was significantly different between tissues from young and old animals. Mean values are shown; vertical lines indicate s.e.mean. *denotes values significantly different from Tyrode-incubated preparations ($P < 0.01$). †denotes values significantly different ($P < 0.01$) from control values (●).

Guinea-pig airways in vivo

Threshold doses of histamine in young and old animals were not significantly different (Table 2). When

Table 1 Response and sensitivity of bronchial preparations from young and old female guinea-pigs: effect of cimetidine

Age	*Treatment*	(*n*)	*Responsiveness*† (g/mm²)	*Sensitivity*‡ (pD_2 value)
Young	None	(9)	0.57 ± 0.09	5.43 ± 0.05
	Post Tyrode	(3)	0.69 ± 0.06	5.62 ± 0.11
	Post cimetidine	(6)	0.59 ± 0.11	5.66 ± 0.09**
Old	None	(10)	0.23 ± 0.06	5.34 ± 0.12
	Post Tyrode	(4)	0.17 ± 0.04	5.49 ± 0.05
	Post cimetidine	(4)	0.31 ± 0.04*	6.06 ± 0.04***

† Tension induced with a maximally effective concentration of histamine.
‡ pD_2 value is the negative logarithm of the molar EC_{50} value for histamine (see Methods).
* Value significantly different from post Tyrode (within age groups).
** Value significantly different from 'none' but not different from post Tyrode (within age groups).
*** Value significantly different from 'none' and post Tyrode (within age groups).
(*n*) Number of tissues examined. Values are mean ± s.e.mean.

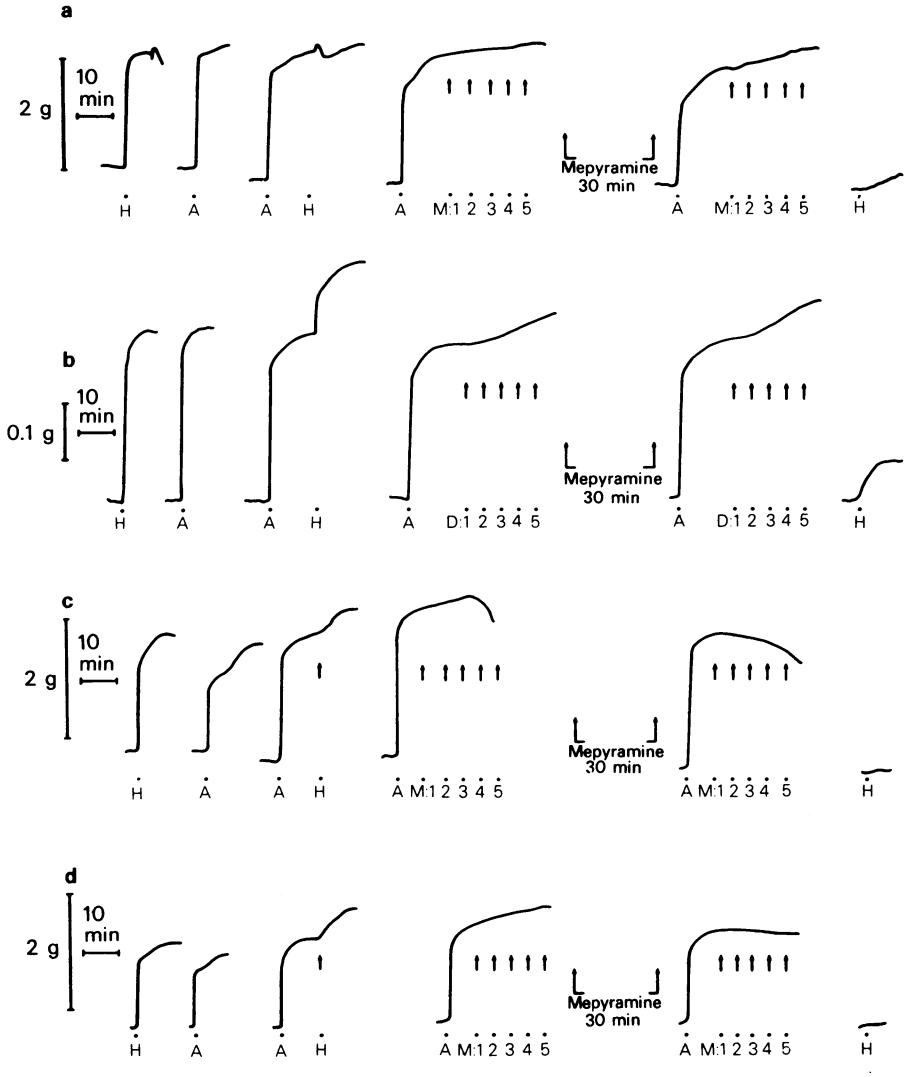

Figure 2 Responses of tracheal and bronchial tissues from young and old guinea-pigs to H_2 agonists. Tissues were exposed to histamine (H, 50 μM) alone or after contraction with acetylcholine (A, 50 μM). After a cycle of approximately 15 min, preparations were again contracted with acetylcholine and 4-methyl histamine (M) or dimaprit (D) was added cumulatively to the bath fluid. The concentrations of 4-methyl histamine were (1) 0.2 mM, (2) 0.4 mM, (3) 0.8 mM and (4) 1.6 mM. The concentrations of dimaprit were the same as for 4-methyl histamine. Preparations were washed, allowed to return to baseline, then were incubated with mepyramine (20 μM) for 30 min followed by washout. Preparations were then examined as previously described. Time and force calibrations are shown. The panels are tracings from (a) a young trachea (b) a young bronchus (c) an old trachea (d) an old bronchus. These tracings are typical recordings from 4 similar experiments.

treated with cimetidine the threshold doses remained unchanged. In young animals, propranolol markedly reduced the histamine threshold doses, i.e. the airways became sensitized to the bronchoconstrictor agent, while in old animals the same dose of propranolol hydrochloride was without effect (Table 1). Cimetidine was without effect after propranolol treatment in young or old guinea-pigs when compared with propranolol treatment alone. In old guinea-pigs, cimetidine significantly reduced airway sensitivity after β-adrenoceptor blockade (Table 2) when compared to threshold doses in vehicle-treated animals.

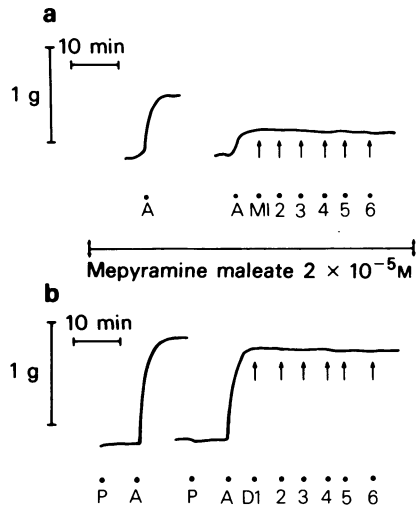

Figure 3 The effect of H_2-agonists upon induced tone in bronchial preparations from female guinea-pigs. Preparations were contracted with a maximally effective concentration of acetylcholine (50 μM). The tissues were then partially contracted with acetylcholine (1–40 μM) in the presence of mepyramine maleate (20 μM). Once the induced tone had reached a plateau either 4-methyl histamine (M1–M6, a) or dimaprit (D1–D6, b) was added as described in Figure 2. The results shown are typical of 4 experiments.

Discussion

If stimulation of H_2-receptor mediates airway muscle relaxation then this physiological process should be demonstrable with dimaprit or 4-methyl histamine. Conversely, the antagonism of H_2-receptors with specific H_2-antagonists (cimetidine and metiamide) should increase the sensitivity of airway preparations to histamine. In this study, neither dimaprit nor 4-methyl histamine relaxed tracheal or bronchial preparations from either young or old female guinea-pigs (Figure 2). These specific H_2-agonists (Durant, Ganellin & Parsons, 1975) were also ineffective after the blockade of H_1-receptors with mepyramine maleate. We have previously reported that H_2-agonists do not relax respiratory tissues from middle aged female guinea-pigs (Duncan *et al.*, 1980; Brink, Ridgway & Douglas, 1980b). The presence of H_2-relaxant receptors in airway muscle preparations has been demonstrated in many species including sheep (Eyre, 1973), cats (Maengwyn-Davies, 1968), dogs (Schneider *et al.*, 1978) rabbits (Fleisch & Calkins, 1976), horses (Chand & Eyre, 1978) and man (Dunlop & Smith, 1977); it is difficult to explain why, in female guinea-pigs at any age, stimulation of H_2-receptors in airway muscle preparations did not induce muscle relaxation. The result is surprising since Okpako *et al.* (1978) have demonstrated that respiratory tissues from guinea-pigs, partially contracted with carbachol, relaxed when exposed to H_2-agonists. In our study, we used concentrations of acetylcholine which induced tones ranging from 20–100% of maximal (Figures 2 and 3). The H_2-agonists, in concentrations that induce elevation of cyclic AMP in tracheae and bronchi (Duncan *et al.*, 1980), did not relax these preparations. Thus, differences in induced tone do not explain why our results differ from those of Okpako *et al.* (1978). We also studied the antagonism of H_2-receptors with an H_2-antagonist, cimetidine. This technique is useful in characterizing receptors since antagonists have a higher affinity for H_2-receptors than agonists. We show that cimetidine significantly enhances the sensitivity of isolated bronchi from old but not young animals (Figure 1). The result suggests that old bronchi have H_2-receptors and their stimulation attenuates histamine-induced contraction. However, bronchial tissues from old guinea-pigs were not relaxed by specific H_2-agonists. Results of our previous studies (Duncan *et al.*, 1980) suggested that H_2-antagonists may have complex effects upon airway muscle. For example, low concentrations of these agents (0.2 μM; 2 μM) depress contractile responses of tracheal tissues from middle aged female guinea-pigs to both histamine and specific H_1-agonists. Higher concentrations of the antagonists (20 μM), sensitize tracheal preparations to the contractile ef-

Table 2 Airway reactivity *in vivo*: the effects of cimetidine and propranolol in young and old female guinea-pigs

	Threshold dose of histamine (% w/v base)†			
Treatment	*Young*	(*n*)	*Old*	(*n*)
Vehicle	0.09 ± 0.01	(9)	0.13 ± 0.01	(4)
Cimetidine	0.12 ± 0.04	(5)	0.19 ± 0.03	(4)
Propranolol	0.03 ± 0.00*	(5)	0.16 ± 0.02	(4)
Propranolol + cimetidine	0.04 ± 0.01**	(4)	0.21 ± 0.04	(4)*

†Threshold dose was determined as described in methods.
(*n*) Number of animals tested. Values are mean ± s.e.mean.
* Value significantly different from threshold dose in animals in same age group treated with vehicle (paired *t* test).
** Value not significantly different from propranolol treatment alone (paired *t* test).

fects of histamine. Bronchial tissues from the same animals always showed enhanced responses to histamine and specific H_1-agonists after H_2-receptor blockade. The absence of a concentration-dependent effect of H_2-antagonists and the significant changes in maximal response (Figure 1; Table 1) are not characteristic of a classical competitive antagonist. Drazen, Venugopalan & Schneider (1980), have also noted that the antagonism of H_2-receptors by metiamide is not concentration-dependent. It is likely that the H_2-antagonists may have other effects (Allan & Eakins, 1978). The absence of H_2-agonist activity or a relaxant activity of histamine *per se* indicate that there are no relaxant H_2-receptors in the tracheae or major bronchi of female guinea-pigs at any age.

Cimetidine (30 mg/kg, i.p.) did not significantly affect the threshold response of airways to histamine in either young or old guinea-pigs (Table 2). In previous studies, H_2-antagonists have been administered in doses of 400 mg orally or 200 mg intravenously in animals (Krell & Chakrin, 1977; Drazen *et al.*, 1979; Ahmed, Eyre, Januszkiewicz & Wanner, 1980). These doses have been sufficient to block H_2-receptors. The absence of an effect of cimetidine *in vivo* indicates that the airway muscle of both young and old female animals has few, if any, physiologically functional H_2-receptors. Drazen *et al.* (1979) have suggested that the relaxant effects of H_2-receptor stimulation is primarily demonstrable in peripheral airways. The data of Yen & Kreutner (1979) support this thesis but their experimental design did not include adequate controls and they used an inappropriate contractile agonist (Brink *et al.*, 1980a; Brink *et al.*, 1981). Our results cast doubt on such an hypothesis since threshold response reflects the sensitivity of peripheral airways to histamine (Douglas *et al.*, 1977).

Major modulators of airway responses to histamine in the guinea-pig are the catecholamines (Douglas, Dennis, Ridgway & Bouhuys, 1973). It seemed possible that they might be masking the effects that H_2-receptor stimulation has on airway muscle function. Thus, we examined the effects of cimetidine in young and old animals after β-blockade. In young guinea-pigs, cimetidine treatment in the presence of β-blockade was still ineffective (Table 2) suggesting that the modulation of histamine-induced bronchoconstriction via H_2-receptor stimulation is of no importance *in vivo*. Propranolol and cimetidine treatments alone tend to reduce airway sensitivity to histamine in old guinea-pigs (Table 2; Douglas *et al.*, 1973). The reduced airway sensitivity seen after administration of both drugs in combination (Table 2) probably reflects a summation of these individual effects.

When examining the effects of cimetidine after β-blockade, we noted an important difference in the effects of propranolol in the different age groups. We examined young and old animals with comparable threshold responses to histamine. While propranolol (10 mg/kg, i.p.) reduced the threshold dose of histamine in young animals it did not affect airway responses to histamine in old guinea-pigs (Table 2). The reason for this difference remains to be determined. Differences in drug distribution probably do not account for this phenomenon because β-blocking agents are actively accumulated in lung tissue (Dollery & Junod, 1976). The data imply that those neural and humoral factors which contribute to airway responses *in vivo* qualitatively change during ontogenesis. Reduced airway responses to histamine, salbutamol, isoprenaline (Brink *et al.*, 1980a) and air pollutants (Schlenker & Jaeger, 1980) during development support this theory.

In summary, this study shows that during ontogenesis there is a development of H_2-receptors, provided one assumes that cimetidine is a specific H_2-antagonist. However, there is no physiological counterpart *in vitro* when H_2-stimulants are examined, nor does airway sensitivity to histamine change *in vivo* after H_2-receptor blockade. We believe that these results indicate that H_2-antagonists may have activities other than simple H_2-antagonism. Therefore, the characterization of H_2-receptors in respiratory tissues will require a complete examination of both H_2-agonists and antagonists before definitive conclusions about histamine receptor subtypes can be made. Significantly, the effects of β-adrenoceptor blockade upon histamine sensitivity are always demonstrable in young guinea-pigs but are never demonstrable in old guinea-pigs. This loss of the effect of propranolol may be related to the decreased sensitivity of airway muscle to catecholamines which occurs during ontogenesis (Brink *et al.*, 1980a) or to a modification in receptor numbers and/or affinity (Douglas, Brink & Duncan, 1981).

Reprint requests to J.S.D. please.

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(Received September 16, 1981.
Revised October 19, 1981.)