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Response and sensitivity of guinea-pig airway muscle preparations to 5-hydroxytryptamine during ontogenesis

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1 Responsiveness ($g\,mm^{-2}$) and sensitivity (pD_2 value) to 5-hydroxytryptamine (5-HT) were markedly reduced in isolated tracheal and bronchial tissues from guinea-pigs during ontogenesis. Responsiveness of the trachea to 5-HT was depressed much more than that to histamine.

2 Airway preparations from young guinea-pigs of either sex always contracted when exposed to 5-HT, an effect that was blocked by methysergide.

3 Airway muscle preparations from old animals exhibited a wide range of responses to 5-HT, namely, no effect, relaxation or contraction.

4 The contractile effect of 5-HT in tracheal and bronchial preparations from old animals was always blocked by methysergide, whereas, the relaxant effect was not.

5 These results indicate that there are significant alterations in the response to 5-HT receptor stimulation in airway muscle preparations from guinea-pigs during ontogenesis.

Introduction

5-Hydroxytryptamine (5-HT) exhibits a wide spectrum of activity in isolated respiratory tissues from different species. A contractile response has been reported in airway muscle preparations from the cat, dog, horse, sheep and calf (Brocklehurst, 1958; Offermeier & Ariens, 1969; Eyre, 1969; Chand & Eyre, 1978). Relaxations are typically observed in similar preparations from the goat (Chand *et al.*, 1979) and man (Mathé *et al.*, 1971; Goldie *et al.*, 1982; Cerrina *et al.*, 1983). In addition, this agonist is inactive on the trachea and bronchus of the rat, rabbit and pig (Chand & Eyre, 1977; Chand & DeRoth, 1978). Since many of these studies were performed in a variety of species of different age and sex it is difficult to know which if any of these parameters may be correlated with the physiological response observed. We decided, therefore, to explore the possibility that this biogenic amine may have different effects in respiratory preparations from both young and old animals of different sex within the same species.

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Methods

Animals

Male and female albino guinea-pigs (Hartley strain, CEFDA, France) were used in this study. Guinea-pigs were divided into two groups based on body weight, namely, young (105 ± 4 g) and old (880 ± 28 g).

Airway tissues in vitro

Tracheal and extrapulmonary bronchial spirals from male and female guinea-pigs were equilibrated in Tyrode solution (composition in 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; pH, 7.4) and gassed with 5% CO_2 in O_2 at 37°C under an initial load of 8 g and 2–3 g, respectively. The high initial loads ensured that, after the 90 min equilibration period, the resting tension was optimal and responses to the agonists were maximal and reproducible. At the end of the equilibration period (90 min), the lengths of the spirals were measured. At the end of the experiments the wet

weights of the preparations were recorded and the tissues were dried in an oven (65°C) for 12 h and weighed. Isometric force-displacement transducers (Narco) and Narco physiographs (MK-IV) were used to record the changes in force.

Concentration-effect curves to 5-hydroxytryptamine

All tissue preparations were first primed with histamine (50 μM). Subsequently a concentration-effect curve was produced by adding graded concentrations of 5-HT in a volume of less than 0.5 ml, in random order, to the tissue bath. When the response to the agonist reached a plateau, the bath fluid was changed for fresh Tyrode solution and the preparations returned passively to their resting tone. Once concentration-effect curves were established, preparations were incubated for 30 min with either vehicle, indomethacin (1.7 μM or 17 μM) or methysergide (0.57 μM or 5.7 μM) and, following washout, a second concentration-effect curve to the agonist was performed.

In some experiments, the effects of 5-HT were assessed in preparations from animals in which tone had been induced by histamine (50 μM). These experiments were performed in the absence and in the presence of methysergide (1 μM).

Calculation of results

EC_{50} values were interpolated by eye from hand-fitted 5-HT concentration-effect curves. These values were transformed into the pD_2 value. The pD_2 value was defined as the negative logarithm of the molar EC_{50} value (the concentration of the agonist producing a contraction which was 50% of maximal contraction). pD_2 values were the measure of the sensitivity of the preparations and the potency of the agonist. The changes in force were directly determined from recordings and were transformed into force/dry weight and force/unit cross-sectional area. The cross-sectional area of the preparations was determined by dividing the tissue wet weight by the tissue length. Responsiveness was expressed as $g\,mm^{-2}$ and was the measure of the efficacy of the agonist. Both maximal response ($g\,mm^{-2}$) and pD_2 values were compared using Student's t test for paired or unpaired variates as appropriate. All data with 5-HT are shown as a percentage of the maximal response ($g\,mm^{-2}$) produced during the generation of the initial concentration-effect curve. The responses to 5-HT subsequent to a 30 min incubation with vehicle or with other drugs were also calculated as a percentage of the initial control maximal response. The results are presented as mean ± s.e.mean.

Drugs

The drugs used and their sources were: histamine dihydrochloride, 5-hydroxytryptamine (5-HT), indomethacin (Sigma Chemical Company, St-Louis, MO, U.S.A.) and methysergide maleate (Sandoz, Basel, Switzerland).

Table 1 Physical characteristics of respiratory muscle preparations from male and female guinea-pigs during ontogenesis

			Length (mm)	*Wet Weight* (mg)	*Dry weight* (mg)
Young	Male (104 ± 8 g)	Trachea (21)	40.33 ± 1.09	49.13 ± 16.25	10.23 ± 0.35
		Bronchus (21)	8.62 ± 0.54	9.61 ± 0.62	1.73 ± 0.09
	Female (106 ± 8 g)	Trachea (6)	38.50 ± 1.41	50.68 ± 2.18	11.37 ± 0.62
		Bronchus (6)	10.17 ± 0.68	12.81 ± 0.62	2.37 ± 0.12
Old	Male (877 ± 20 g)	Trachea (24)	55.21 ± 3.22	433.75 ± 25.58	132.39 ± 8.60
		Bronchus (24)	16.68 ± 0.73	84.79 ± 11.74	28.37 ± 4.71
	Female (922 ± 87 g)	Trachea (14)	58.60 ± 3.16	440.56 ± 15.57	158.46 ± 6.93
		Bronchus (14)	18.20 ± 1.97	98.57 ± 14.81	38.56 ± 7.39

At the end of the 90 min equilibration period the lengths of the muscle preparations were measured. Tissues were weighed at the end of the experiment and again after drying at 65°C for 12 h. The numbers in parentheses represent the number of preparations. Values are the mean ± s.e.mean.

Table 2 The maximal response to histamine and 5-hydroxytryptamine (5-HT) in isolated respiratory muscle preparations from male and female young guinea-pigs

	Preparation	*Histamine* ($g\,mm^{-2}$)	*5-HT* ($g\,mm^{-2}$)	*5-HT* (pD_2 value)
Young male (119 ± 8 g)	Trachea	1.08 ± 0.12 (9)	0.77 ± 0.08[a] (24)	6.77 ± 0.05 (24)
	Bronchus	0.84 ± 0.22 (9)	0.27 ± 0.05 (24)	6.66 ± 0.05 (24)
Young female (106 ± 9 g)	Trachea	0.95 ± 0.16 (6)	0.56 ± 0.09[a] (6)	6.68 ± 0.11 (6)
	Bronchus	0.42 ± 0.12 (6)	0.15 ± 0.06 (6)	6.55 ± 0.10 (6)

The force induced with a maximally effective concentration of histamine or 5-HT was recorded and transformed into force/cross-sectional area ($g\,mm^{-2}$).
Concentration-effect curves to 5-HT were produced in both tracheal and bronchial preparations and pD_2 values were interpolated from individual curves. Values are the mean ± s.e.mean. The numbers in parentheses represent the number of preparations.
[a] Indicates values significantly different from results in bronchial preparations ($P < 0.05$).

Results

The physical characteristics of the different types of airway preparations used are presented in Table 1. These parameters were necessary for the normalization of the force induced by both histamine and 5-HT in tissues from each age group. When the force was corrected for tissue size, the maximal response ($g\,mm^{-2}$) to both histamine and 5-HT in respiratory tissues from young guinea-pigs (male and female) was greater than those observed in the same types of preparations from older animals (Tables 2 and 3). In tissues from young guinea-pigs of either sex, the maximal response ($g\,mm^{-2}$) to 5-HT was different when data from tracheal preparations were compared to bronchial tissue results. However, there was no difference when the same preparations were compared between male and female animals in this age group. There was a tendency for bronchial preparations to be less responsive to histamine than tracheal preparations from young animals whether male or female. The contractile response to histamine in these preparations was also similar within this age group. Airway preparations therefore, from animals of either sex within each age group were comparable. The ratio of the maximal response of 5-HT to that of histamine in

Table 3 The maximal response to histamine and 5-hydroxytryptamine (5-HT) in isolated respiratory muscle preparations from male and female old guinea-pigs

	Preparation	*Histamine* ($g\,mm^{-2}$)	*5-HT* ($g\,mm^{-2}$)	*5-HT* (pD_2 value)
Old male (940 ± 42 g)	Trachea	0.32 ± 0.05 (11)	0.06 ± 0.01 (11)	5.26 ± 0.12 (4)
	Bronchus	0.10 ± 0.02 (11)	0.04 ± 0.01 (11)	5.40 ± 0.06 (4)
Old female (918 ± 74 g)	Trachea	0.28 ± 0.02 (6)	0.04 ± 0.01 (6)	ND
	Bronchus	0.21 ± 0.07 (6)	0.04 ± 0.02 (6)	ND

The force induced with a maximally effective concentration of histamine or 5-HT was recorded and transformed into force/cross-sectional area ($g\,mm^{-2}$). Concentration-effect curves to 5-HT were produced in both tracheal and bronchial tissues and pD_2 values were interpolated from individual curves. Values are the means ± s.e.mean. The numbers in parentheses represent the number of preparations. ND indicates values not determined.

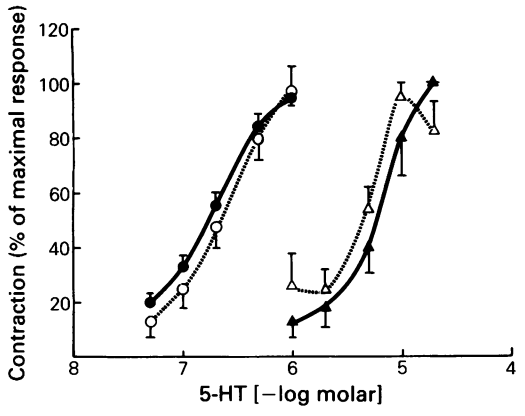

Figure 1 The effects of 5-hydroxytryptamine (5-HT) in isolated airway muscle preparations from male guinea-pigs of different ages: tracheal preparations from young animals (●) and old (▲) as well as bronchial preparations from these same guinea-pigs (young: ○ and old △). The data presented are the initial curves produced in the preparations (young: $n = 24$ and old: $n = 4$). Values are means with vertical lines showing s.e.mean.

tracheal preparations from both male and female guinea-pigs was reduced during ontogenesis (0.71 to 0.18 and 0.59 to 0.14 respectively). However, bronchial preparations did not exhibit this reduction when a similar comparison was made between the two age groups (0.32 to 0.40 and 0.35 to 0.19 respectively).

Concentration-effect curves to 5-HT in preparations from both young and old male animals are shown in Figure 1. Airway tissues from older animals were significantly ($P < 0.05$) less sensitive to 5-HT (compare pD_2 values, Tables 2 and 3). Tracheal and bronchial preparations from animals of either sex within the young age group were similar. 5-HT concentration-effect curves were not determined in tissues from old female guinea-pigs because of the extremely small response in these tissues. Tracheal preparations from young male guinea-pigs which were treated with a high concentration of indomethacin (17 μM) were less responsive and less sensitive to 5-HT. However, at a lower concentration (1.7 μM) we always observed an increased maximum response despite reduced sensitivity to 5-HT (Figure 2). Bronchial tissues from these same male animals exhibited an increased responsiveness in the presence of indomethacin at either concentration (Figure 2). We did not examine systematically the effects of indomethacin in respiratory tissues from old male guinea-pigs because of the small response to 5-HT in these tissues (Table 3); however, in two experiments, tracheal and bronchial preparations exhibited a 1.4 fold increase in response subsequent to a 30 min incubation with indomethacin (17 μM). Methysergide (1 μM) completely blocked the

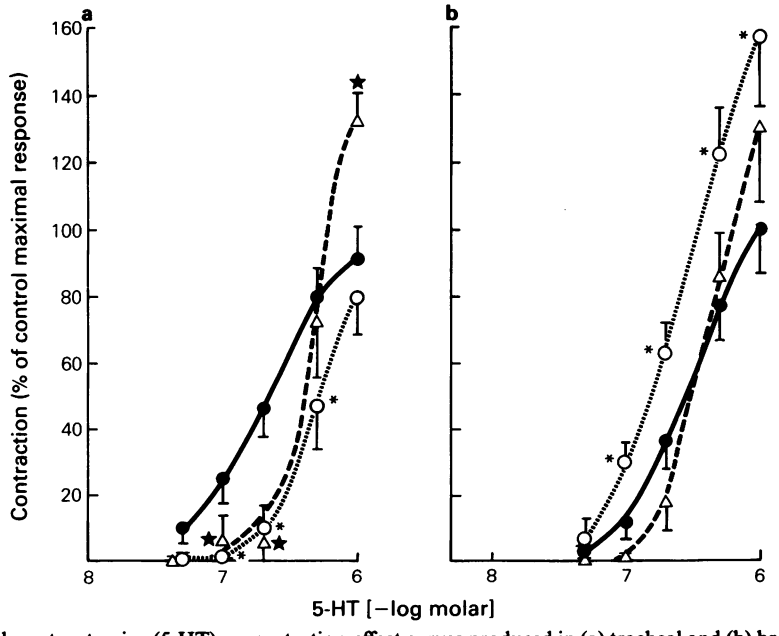

Figure 2 5-Hydroxytryptamine (5-HT) concentration-effect curves produced in (a) tracheal and (b) bronchial tissues from young male guinea-pigs. Responses to 5-HT after a 30 min incubation with Tyrode solution (controls: ●; $n = 12$) or indomethacin (1.7 μM: △; $n = 6$ or 17 μM: ○; $n = 6$). Values are mean with vertical lines showing s.e.mean. ★ and * indicate those values which were significantly different from controls ($P < 0.05$).

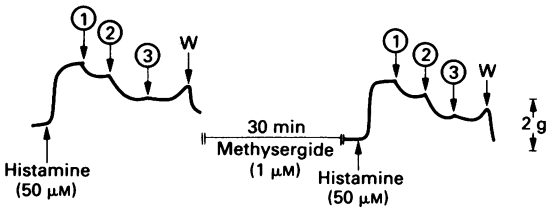

Figure 3 The effect of 5-hydroxytryptamine (5-HT) in an isolated tracheal preparation from an old male guinea-pig in which tone was induced with a maximally effective concentration of histamine (50 μM). Three concentrations of 5-HT were added to the contracted tissue, at (1) 1 μM; (2) 10 μM; and (3) 100 μM. Subsequent to a 30 min incubation with methysergide (1 μM) the procedure was repeated.

contractile response to 5-HT in both types of preparations from the different age groups.

In tracheal preparations from 2 out of 9 old male guinea-pigs 5-HT (100 μM) elicited a small ($0.02\,g\,mm^{-2}$) relaxation of basal tone. A similar relaxant effect ($0.03 \pm 0.02\,g\,mm^{-2}$) was observed in tracheal preparations from 4 out of 10 old female guinea-pigs. Bronchial preparations from these same male and female guinea-pigs did not exhibit relaxation to 5-HT. When 5-HT was added to tracheal and bronchial tissues from old male animals, where tone was induced with a maximal effect concentration of histamine (50 μM), a relaxation occurred only in preparations taken from 3 out of 13 animals and we were unable to block this relaxant effect with methysergide (Figure 3). We did not observe this relaxant effect in airway preparations from young animals of either sex.

Discussion

Tracheal and bronchial preparations from old guinea-pigs were less responsive and approximately 10 fold less sensitive to 5-HT than similar tissues from younger animals. The ratio of the maximal response to 5-HT to that to histamine was considerably reduced in tracheal preparations during ontogenesis despite a decrease in the maximal response to histamine in preparations from old animals, a phenomenon that has already been documented (Brink *et al.*, 1980). These results suggest that in the trachea the response to 5-HT receptor stimulation and/or the receptor coupled system has been altered during aging. Bronchial preparations do not exhibit similar changes in the 5-HT/histamine ratio indicating that there may be important differences between tracheal and bronchial preparations from the same animal.

Airway muscle preparations from young animals always contracted to 5-HT and the concentration-effect curves were modified by indomethacin or completely blocked by methysergide. These data indicate that respiratory tissues from young guinea-pigs contain 5-HT receptors probably of the D-type and that during the contractile response to this agonist prostaglandins may be released which modulate the response. In airway tissues from old guinea-pigs 5-HT had a smaller and more variable effect. In some tissues it caused contraction whilst in others it induced a methysergide-resistant relaxation which may be similar to the relaxant response observed in human isolated bronchial muscle preparations, an effect which is also not blocked by methysergide (Cerrina *et al.*, 1983).

Our results demonstrate that important changes may occur with regard to 5-HT receptor stimulation and/or its effector systems during aging, although the mechanisms involved remain to be elucidated.

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