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Age and region-dependent contraction to α-adrenoceptor agonists in rat and guinea-pig isolated trachea

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1 The influence of age and of region on α-adrenoceptor-mediated contraction to (−)-adrenaline and (−)-noradrenaline was examined in rat (4–136 weeks) and guinea-pig (2–156 weeks) isolated tracheal ring preparations with particular emphasis on the early (up to 12 weeks) maturation phase.

2 In rat tracheal rings, significant regional variation was observed with respect to maximal (−)-adrenaline-induced contraction, such that the greatest activity was seen in ring preparations from the laryngeal end of the trachea. Tracheal rings from the carinal end responded very poorly or were unresponsive to (−)-adrenaline, depending on animal age. These regional differences were seen across the age range. The potencies of (−)-adrenaline and (−)-noradrenaline remained unchanged with respect to animal age, but the maximum contractile tension that developed in response to these agonists increased with increasing animal age in all regions of the trachea.

3 In guinea-pig isolated tracheal tissue, maximum contractile responses (E_{max}) to (−)-adrenaline and (−)-noradrenaline remained unchanged with increasing animal age. In addition, there was no evidence for a region-dependence in the responsiveness of tracheal tissue to α-adrenoceptor-mediated contraction in this species.

4 In both guinea-pig and rat isolated tracheal tissue, α-adrenoceptor-mediated contraction appeared to involve the activation of α_1-adrenoceptors.

Keywords: Age; α-adrenoceptors; trachea; regional variations

Introduction

α-Adrenoceptor-mediated contraction of airway smooth muscle has been described in the dog (Barnes *et al*., 1983), guinea-pig (Fleisch *et al*., 1970; Takayanagi *et al*., 1990; 1991), cat (Miura *et al*., 1988), rabbit and in older rats (Fleisch *et al*., 1970), although little is known about the role and subtype identification of this receptor. Current evidence suggests that α_2-adrenoceptors are primarily responsible for mediating (−)-noradrenaline-induced contraction in guinea-pig (Takayanagi *et al*., 1990) and canine (Barnes *et al*., 1983) airway smooth muscle.

Whilst various studies have focused on the effects of disease states such as asthma on contractile function mediated via α-adrenoceptors (Kneussel & Richardson, 1978; Szentivanyi, 1979; 1980; Goldie *et al*., 1985; Spina *et al*., 1989), little research has been directed towards determining the effects of ageing in the normal functioning of these systems.

Age-related changes in α-adrenoceptor-mediated function have been observed in rat vas deferens (Docherty & O'Malley, 1983) and vascular preparations from rat (Docherty & Hyland, 1986; Docherty, 1988; Takayanagi *et al*., 1989; Gurdal *et al*., 1995) and dog (Toda & Shimizu, 1987). In contrast, no age-related change in α-adrenoceptor-mediated responses were seen in human vascular tissue (Scott & Reid, 1982; Stevens *et al*., 1982) or rat tail arteries (Tsai *et al*., 1993). In airway tissue, Fleisch and coworkers (1970) observed a significant difference in α-adrenoceptor-mediated contraction between young and old rats, while more recently, an age-dependent decline in guinea-pig tracheal contraction to noradrenaline has been described (Takayanagi *et al*., 1991). Such studies have utilized relatively narrow age ranges outside the period of early animal maturation. It was therefore of interest to determine whether these effects were part of a continuous age-related trend. Accordingly, α-adrenoceptor-mediated contraction was examined in airway tissue from rats and guinea-pigs over an age range encompassing both the early maturation phase and the adult period of the life cycle.

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Methods

Functional studies

Male guinea-pigs (SR/C tricolour aged 2–156 weeks; male Hartley aged 12 weeks) and male Wistar rats (aged 4–136 weeks) were used in this study. Guinea-pigs were killed by cervical dislocation and exsanguinated; rats were killed by a blow to the head followed by exsanguination. Tracheal rings 2–3 mm in width were mounted in Krebs-bicarbonate buffer (composition (mM): NaCl 117, $NaHCO_3$ 25.0, KCl 5.36, KH_2PO_4 1.03, $MgSO_47H_2O$ 0.57, $CaCl_22H_2O$ 2.5 and glucose 11.1) in 20 ml water-jacketed organ baths gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Preparations were attached to an isometric transducer (Grass, FT03) coupled to a preamplifier and Rikadenki recorder (model 1328L). Tension of 500 mg weight was applied and the tissue allowed to equilibrate for 1 h with regular changes of the bathing solution. Spontaneous increases or decreases in resting tone were compensated for by readjusting to 500 mg weight tension. Following the 1 h equilibration period, tracheal preparations were contracted with 3.2 μM carbachol to assess tissue viability. After washout and a 30 min rest period, this challenge was repeated. After a further washout and rest period, two sequential cumulative concentration-effect curves were constructed, the first to carbachol and the second to either (−)-adrenaline, (−)-noradrenaline, (−)-phenylephrine, methoxamine or clonidine in the presence of (±)-propranolol

(10 μM). This concentration of (±)-propranolol has previously been demonstrated to be effective in blocking β-adrenoceptor agonist-mediated relaxation of guinea-pig tracheal tissue (Weissberg *et al*., 1977; Brandt & Meyer, 1987).

The potency ($pEC_{50} = -\log_{10}EC_{50}$, where EC_{50} represents the concentration of agonist required to elicit 50% of the maximal response) of each agonist was determined from each tracheal ring. The maximum contractile response (E_{max}) of the tracheal ring tissue in response to the α-adrenoceptor agonists was calculated as a percentage of carbachol-induced maximum contraction (CCh E_{max}), determined from the cumulative concentration-effect curve in the same tissue preparation. This was done to standardize for increases in E_{max} resulting from age-related increases in muscle mass. Tracheal preparations from guinea-pig and rats of all ages tested, contracted in response to carbachol in a concentration-dependent manner. Carbachol potency (pEC_{50}) remained essentially constant with increasing age, whereas E_{max} increased significantly with increasing age, presumably reflecting age-dependent increases in muscle mass. Confirming this are data (Table 1) illustrating the increase in carbachol E_{max} with respect to animal age in both guinea-pig and rat isolated tracheal tissue. Similarly, the E_{max} for the non-receptor agonist, potassium (K^+) also increased ($P<0.05$; 1-way ANOVA) with respect to animal age and the ratio of carbachol to K^+ E_{max} values remained unchanged ($P>0.05$; 1-way ANOVA) as a function of animal age (Table 1). Accordingly, all further contractile responses to α-adrenoceptor agonists were calculated as % carbachol E_{max}. Carbachol was chosen rather than K^+ as contractile responses to K^+ were not as stable in terms of maintenance of the contractile response, particularly in tracheal tissue from very young animals. In studies in which the effect of (±)-propranolol and/or indomethacin pretreatment on responses to (−)-adrenaline was examined, contraction was assessed as % (−)-adrenaline E_{max}.

The potency of the non-selective α-adrenoceptor antagonist phentolamine (0.1–3 μM) against noradrenaline in guinea-pig isolated tracheal preparations was determined from Schild plots (Schild, 1947, 1949). Concentration-effect curves for (−)-noradrenaline were constructed in each preparation. Preparations were then washed to re-establish baseline tone and rested for 30 min before exposure to phentolamine for 30 min. A second concentration-effect curve to (−)-noradrenaline was then constructed in each preparation. Thus, four of the twelve tracheal preparations served as time controls, with two other groups of four preparations exposed to 0.1 or 0.3 μM phentolamine. The tissues were then washed after construction of noradrenaline concentration-effect curves to re-establish baseline tone and rested for 30 min before exposure to 10 fold higher concentrations (1 and 3 μM) of phentolamine, respectively. All of these experiments were conducted in the presence of 10 μM (±)-propranolol.

Data analysis

pEC_{50} values for each cumulative concentration-effect curve were estimated by fitting the data to a sigmoidal function by means of an iterative, non-linear, least squares regression analysis programme based on the downhill simplex method for function minimization (Nelder & Mead, 1965; Press *et al*., 1986). Maximum contractile response (E_{max}) was also measured. Mean values for animals within each age group were then combined to give a final mean estimate (±s.e.mean) of pEC_{50} and E_{max} values for each age group within each species. For cumulative concentration-effect curves to methoxamine, true maximum response as indicated by plateau at the highest concentrations used was not always obtained. Thus apparent pEC_{50} values were determined from curves constructed after E_{max} had been defined as the response to the highest concentration used.

Mean data were examined by one-way analysis of variance (ANOVA) and treatment effects considered different if $P<0.05$. For studies involving Schild analysis, pA_2 and slope estimates were obtained from a computer-fitted linear regression analysis. For each animal, pA_2 and slope estimates were obtained and the mean values (±s.e.mean) for these parameters were calculated from the combined means of all animals.

Drugs

Drugs used were (−)-adrenaline bitartrate, carbamylcholine chloride (carbachol), clonidine hydrochloride, indomethacin, (−)-noradrenaline bitartrate, (−)-phenylephrine hydrochloride (Sigma Chem. Co., U.S.A.), potassium chloride (analytical grade) (Fluka AG), phentolamine mesylate (Ciba-Geigy, Australia), (±)-propranolol hydrochloride (ICI, Australia), methoxamine hydrochloride (Burroughs Wellcome Co., U.S.A.).

All drug solutions were prepared daily. Stock solutions and dilutions of sympathomimetic amines were prepared in 20 μg ml^{-1} ascorbic acid saline. Stock solutions of indomethacin were prepared in 5% (w/v) $NaHCO_3$ with dilutions made in 0.9% (w/v) NaCl solution (saline). All other drugs were prepared in saline.

Results

Region- and age-dependent α-adrenoceptor-mediated contraction

Rat trachea In the presence of (±)-propranolol (10 μM), isolated tracheal smooth muscle preparations obtained from

Table 1 Potency (pEC_{50}) and E_{max} responses to carbachol and potassium (K^+) with respect to animal age and the effects of normalization of carbachol E_{max} with potassium E_{max}

	Animal		K^+		*Carbachol*		
Species	*age*	n	pEC_{50}	E_{max} (mg)	pEC_{50}	E_{max} (mg)	E_{max} (% K^+)
Guinea-pig	2	3	1.84±0.03	317.4±14.9	6.89±0.04	330.7±55.2	111.6±16.5
	6	5	1.76±0.03	411.3±17.0	6.75±0.05	461.1±32.9	130.6±1.4
	12	6	1.71±0.03	512.2±95.3	6.67±0.04	531.8±24.4	134.0±14.0
	26	3	1.73±0.06	954.4±86.2	6.65±0.08	917.2±70.5	98.9±16.6
Rat	4	7	1.50±0.02	149.3±23.4	6.17±0.04	286.7±19.6	197.7±13.7
	8	4	1.45±0.02	301.6±57.1	6.30±0.03	286.2±61.5	93.3±6.8
	12	5	1.47±0.01	316.8±42.5	6.35±0.03	428.0±49.6	137.2±7.2
	16	4	1.48±0.02	291.7±36.9	6.37±0.05	434.0±39.4	151.0±6.4

all regions of the trachea of young rats (4 and 8 weeks of age) failed to contract in response to (−)-adrenaline, (−)-noradrenaline, (−)-phenylephrine, methoxamine or clonidine, but contracted strongly to carbachol in a concentration-dependent manner. In contrast, tissue preparations from older rats, primarily from the laryngeal region of the trachea, contracted in a concentration-dependent manner in response to the non-selective α-adrenoceptor agonists, (−)-adrenaline and (−)-noradrenaline, but not to (−)-phenylephrine, methoxamine or clonidine. The pEC_{50} values for (−)-adrenaline and (−)-noradrenaline in isolated tracheal smooth muscle tissue from 56 week old animals were 5.33 ± 0.04 ($n=3$) and 5.16 ± 0.06 ($n=3$) respectively and the maximum contractile responses (E_{max}) were $16.5 \pm 2.2\%$ of CCh E_{max} ($n=3$) and $12.6 \pm 2.2\%$ of CCh E_{max} ($n=3$), respectively. Similar contractile responses were observed to (−)-adrenaline (Figure 1) ($pEC_{50} = 5.50 \pm 0.15$; $E_{max} = 10.6 \pm 1.5\%$ of CCh E_{max}; $n=3$) and (−)-noradrenaline ($pEC_{50} = 5.04 \pm 0.14$; $E_{max} = 6.0 \pm 1.0\%$ CCh E_{max}; $n=3$) in the absence of (±)-propranolol. In the presence of the cyclo-oxygenase inhibitor indomethacin (5 μM), no significant changes in the pEC_{50} or E_{max} of (−)-adrenaline were observed (Figure 1).

The degree of contractile response obtained to the α-adrenoceptor agonists varied markedly with tracheal region, with only weak contractile responses obtained in preparations from the mid or carinal regions of the trachea. The greatest maximal responses (E_{max}) were also obtained from preparations taken from the laryngeal end of the trachea, with progressively lower E_{max} values obtained from preparations taken in sequence towards the bronchial, i.e. carinal end. Tracheal rings from the carinal region of the trachea from rats up to 56 weeks of age failed to contract in response to either (−)-adrenaline or (−)-noradrenaline in the absence or presence of (±)-propranolol, although weak contraction was seen in such preparations from 128 week old animals.

The ability of various regions of the rat trachea to contract in response to both (−)-adrenaline and (−)-noradrenaline were age-dependent. In laryngeal rings which contracted more actively to (−)-adrenaline and (−)-noradrenaline than mid or carinal preparations, these agents were virtually inactive in tissue from animals aged 4 or 8 weeks. In these young animals, no contraction was observed in the mid or carinal regions of the trachea, whereas in older rats (12 weeks or above), contraction was regularly observed in the mid trachea and occasionally in the lower, carinal region. In animals aged 16 weeks, contractile responses to (−)-adrenaline in the presence of (±)-propranolol were observed in the laryngeal and mid regions of the trachea, but not in the lower half of the trachea. In contrast, in tracheal tissue from 128 week old rats, contractile responses to (−)-adrenaline were observed in all sections of the trachea, with a significant decline in maximal contraction occurring with increasing distance from the laryngeal region of the trachea (Figure 2). The contractile responsiveness of the trachea to (−)-adrenaline was significantly greater in older animals than in younger animals and this was also dependent on the region of the trachea from which tissue was examined (Figures 2 and 3). Despite the differences in E_{max} along the trachea, where the potencies of (−)-adrenaline and (−)-noradrenaline could be accurately assessed (i.e. 16 weeks and older), they were neither age- nor region-dependent (Figure 4).

Guinea-pig trachea In the absence of (±)-propranolol, both (−)-adrenaline and (−)-noradrenaline caused concentration-dependent relaxation of the tracheal smooth muscle at low concentrations (0.2–3.2 μM). This was overcome in the presence of higher concentrations of agonist (greater than 10 μM) to produce concentration-dependent contraction (data not shown). In the presence of (±)-propranolol (10 μM), contraction of guinea-pig isolated tracheal tissue was seen in response to (−)-adrenaline ($pEC_{50} = 6.34 \pm 0.17$; $E_{max} = 39.8 \pm 16.1\%$ of CCh E_{max}; $n=5$) and (−)-noradrenaline ($pEC_{50} = 6.09 \pm 0.16$; $E_{max} = 37.7 \pm 9.7\%$ of CCh E_{max}; $n=5$) (Figure 5). In the presence of indomethacin (5 μM), contractile responses to (−)-adrenaline and (−)-noradrenaline in guinea-pig tracheal rings were completely abolished. The α_1-adrenoceptor-selective agonist methoxamine also caused contraction with an apparent pEC_{50} of 4.47 ± 0.10 ($E_{max} = 39.6 \pm 10.1\%$ CCh E_{max}; $n=5$), although the concentration-effect relationship was not assessed at concentrations

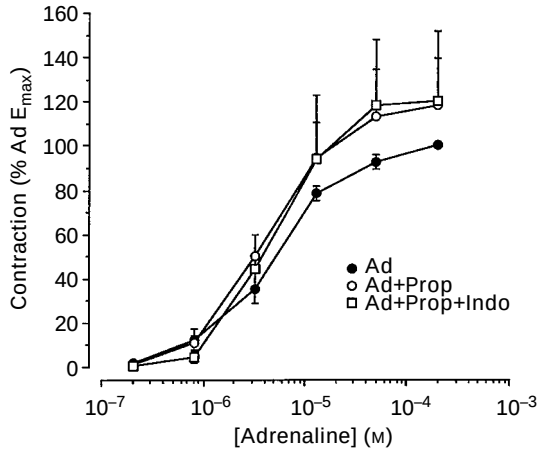

Figure 1 Cumulative concentration-effect curves to (−)-adrenaline (Ad) in the absence (control) and presence of (±)-propranolol (Prop; 10 μM) and in the presence of (±)-propranolol and indomethacin (Indo; 5 μM) in rat isolated tracheal tissue. Points represent mean estimates from $n=5$ animals aged 56 weeks, with vertical lines indicating s.e.mean.

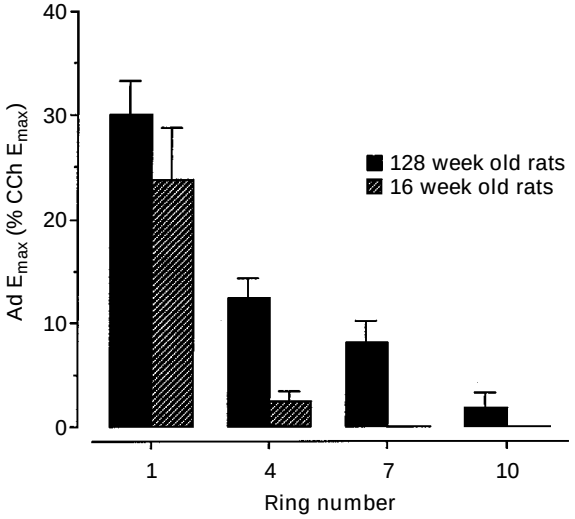

Figure 2 Influence of tracheal region on the maximum contractile tension induced by (−)-adrenaline (Ad) in rat isolated tracheal rings. Ring number indicates the position of the tracheal preparation in relation to the whole trachea, with 12 rings per trachea, number 1 originating from the laryngeal region of the trachea, and number 12 originating from the carinal region. Data presented as the mean of 3 animals with vertical lines representing the s.e.mean. CCh : carbachol.

greater than 200 μM. Weak contraction to (−)-phenylephrine was also observed. In contrast, the α_2-adrenoceptor-selective agonist clonidine induced very weak if any contraction (Figure 5). The potency (pEC_{50}) order for the α-adrenoceptor agonists was (−)-adrenaline > (−)-noradrenaline > methoxamine in the ratio 1 : 1.8 : 74.1. In the Hartley strain of guinea-pigs, a similar profile of responses to these α-adrenoceptor agonists was observed, including the failure of clonidine to cause significant levels of contraction (data not shown).

In guinea-pigs of all ages, both (−)-adrenaline and (−)-noradrenaline caused concentration-dependent contraction in preparations from all tracheal regions and there was no evidence of a region-dependence in the responsiveness of guinea-pig isolated tracheal tissue to these α-adrenoceptor agonists. During the maturation phase of animal growth (2–12 weeks), the potencies of (−)-adrenaline and (−)-noradrenaline tended to increase, peaked at 12 weeks, then tended to decline

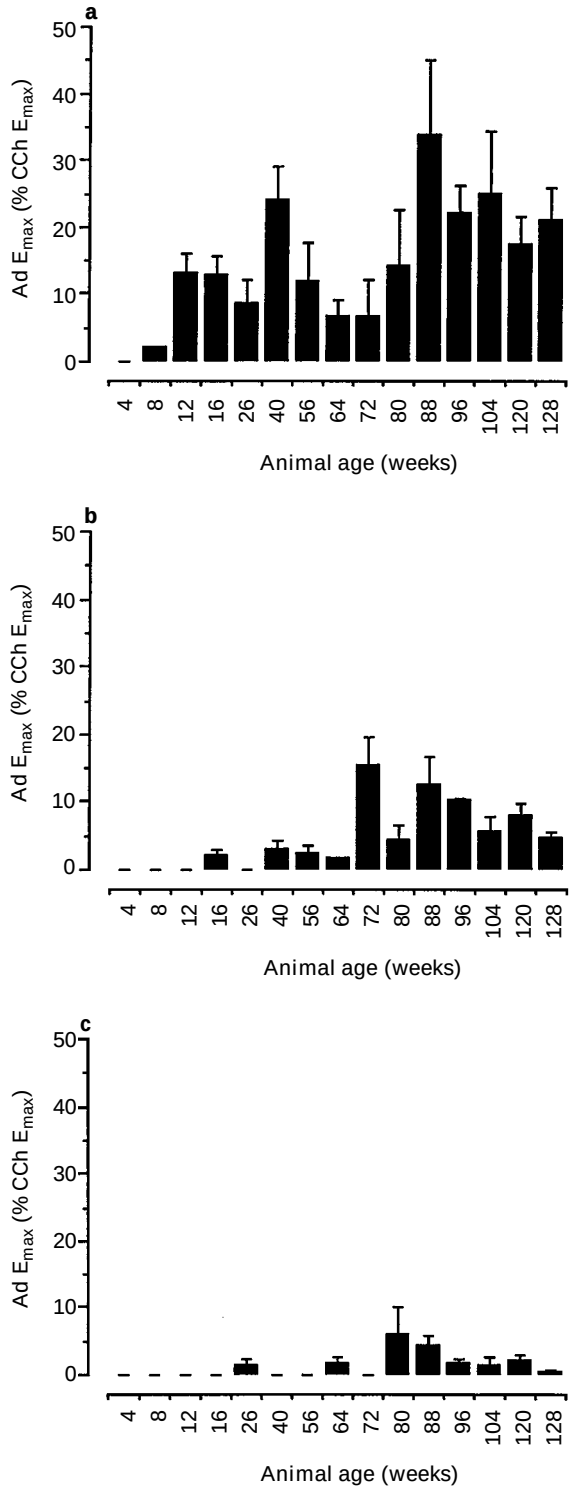

Figure 3 Influence of animal age on the maximal contractile response (E_{max}) of rat isolated tracheal tissue to (−)-adrenaline (Ad). (a) Laryngeal region of trachea (ring numbers 1, 2, 3 and 4 of 12 rings), (b) middle region (rings 5, 6, 7 and 8 of 12 rings) and (c) carinal region (rings 9, 10, 11 and 12 of 12 rings). Data presented as the mean from 3–4 animals with vertical lines representing the s.e.mean.

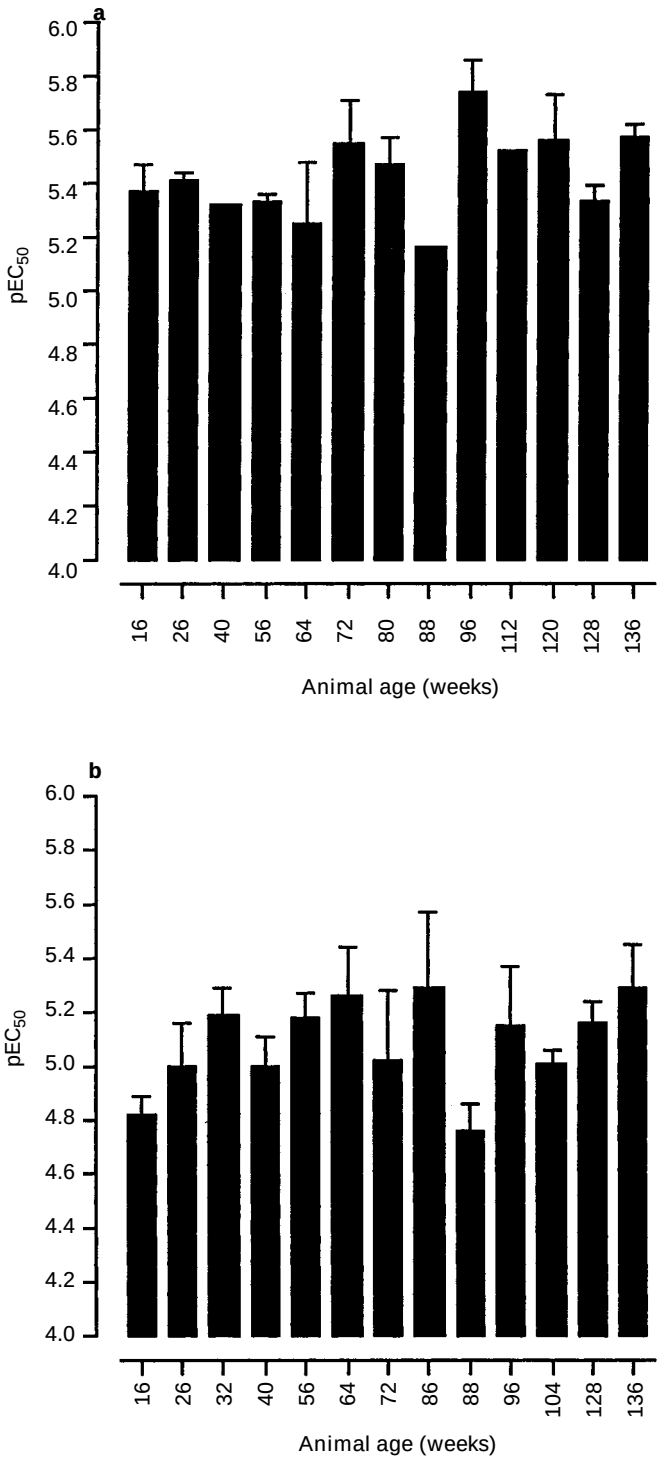

Figure 4 Influence of age on the potency (pEC_{50}) of (a) (−)-adrenaline and (b) (−)-noradrenaline in rat isolated tracheal tissue with respect to animal age. Data represented as the mean of 3–4 animals at each age group, with vertical lines indicating the s.e.mean.

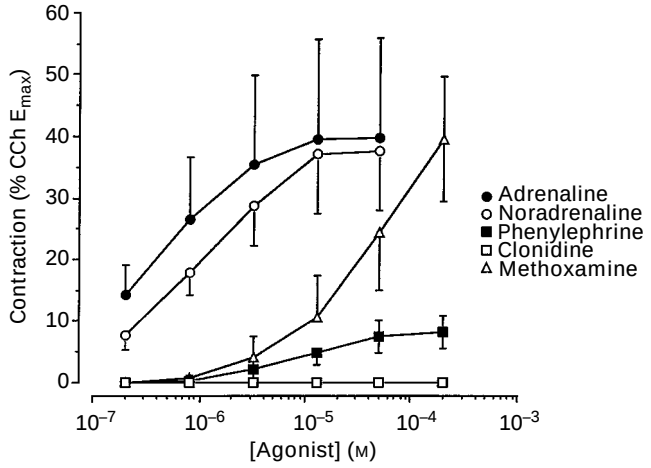

Figure 5 Cumulative concentration-effect curves to (−)-adrenaline ($n=5$ animals), (−)-noradrenaline ($n=5$), (−)-phenylephrine ($n=3$), clonidine ($n=4$) and methoxamine ($n=5$) in guinea-pig isolated trachea. Points represent the mean of estimates from animals aged 6 weeks with vertical lines indicating the s.e.mean.

by 26 weeks of age, after which the potencies remained essentially constant as a function of animal age (Figure 6a and b). Despite these tendencies, statistical analysis by use of ANOVA, indicated that no significant difference ($P<0.05$) in the potencies of (−)-adrenaline or (−)-noradrenaline over the maturation phase of growth (2–12 weeks) or over the period 2–26 weeks. In addition, ANOVA of the E_{max} data for the maximal contractile responses of the tracheal preparations in response to (−)-adrenaline or (−)-noradrenaline over the maturation period (2–12 weeks) showed no significant changes ($P>0.05$) with respect to animal age. Furthermore, assessments across the entire age range indicated no significant age-associated change in the potencies or E_{max} values for these spasmogens (ANOVA, $P>0.05$) (Figure 6).

(−)-Noradrenaline-induced contraction of guinea-pig isolated tracheal tissue in the presence of 10 μM (±)-propranolol was inhibited in a concentration-dependent manner by the non-selective α-adrenoceptor antagonist phentolamine. pA_2 and Schild slope values for phentolamine against (−)-noradrenaline as the contractile agonist, were 6.91 ± 0.14 and 0.98 ± 0.07 ($n=5$), respectively (Figure 7).

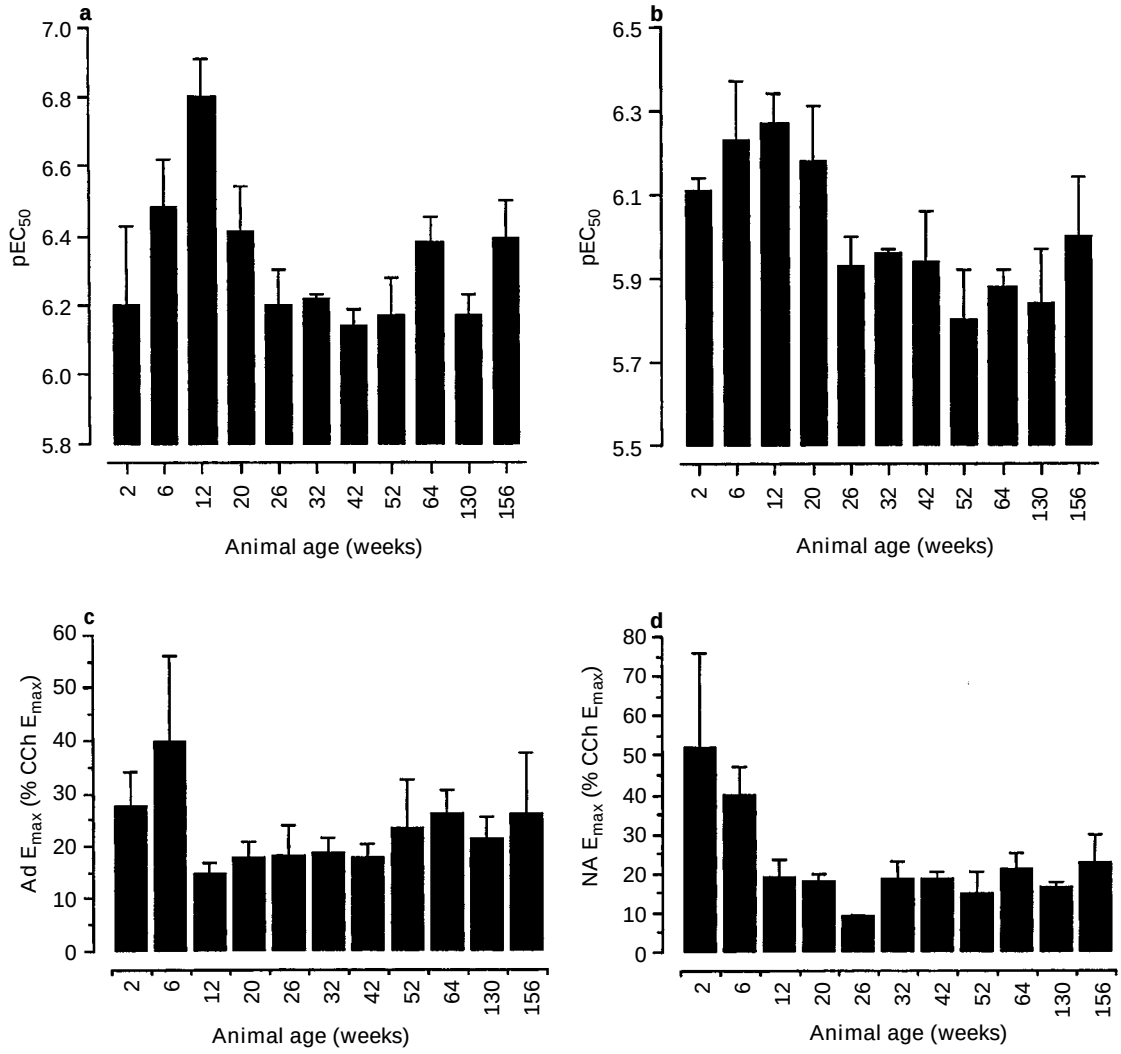

Figure 6 Influence of age on (−)-adrenaline (Ad) (a) potency (pEC_{50}) and (c) maximal contractile effect (E_{max}, measured as % of carbachol E_{max}) and on (−)-noradrenaline (NA) (b) potency (pEC_{50}) and (d) maximal contractile effect (E_{max}, measured as % of carbachol E_{max}) in guinea-pig isolated tracheal smooth muscle. Data represented as the mean of 3–6 animals at each age group, with vertical lines indicating the s.e.mean.

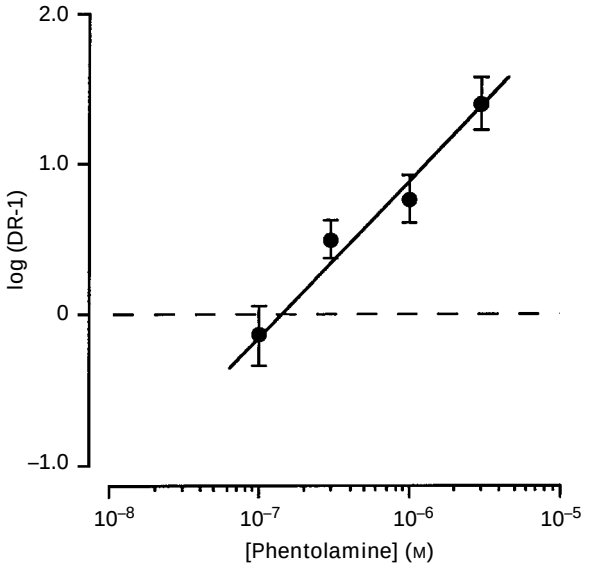

Figure 7 Schild plot for the non-selective α-adrenoceptor antagonist phentolamine against noradrenaline-induced contraction of guinea-pig isolated tracheal smooth muscle. Values are presented as the mean data from 5 animals with vertical lines representing the s.e.mean.

Discussion

In this study we have demonstrated significant age- and region-related changes in α-adrenoceptor-mediated contraction in rat isolated trachea which were not apparent in guinea-pig trachea. Maximal contractile responses to α-adrenoceptor agonists were greatest in the laryngeal region of the rat trachea, with responsiveness decreasing towards the carinal region, where contraction was rarely observed. With increasing animal age, the ability of tracheal tissue to contract to α-adrenoceptor agonists extended further down the trachea, such that some carinal preparations from 104–128 week old animals were responsive, although contractile responses in these preparations were extremely small. In contrast to the rat, no regional differences in the responsiveness of guinea-pig isolated trachea to α-adrenoceptor agonists were observed.

Similar regional variations in tracheal responsiveness have also been described in canine trachea to the muscarinic agonist methacholine and the β-adrenoceptor agonist isoprenaline, with greater potency and maximal effects observed in the laryngeal region of the trachea than in the lower, carinal region (Minneman *et al*., 1983; Moore *et al*., 1986). To our knowledge, there are no published data on regional differences within the trachea with respect to muscarinic agonist- or β-adrenoceptor agonist-induced responses in the guinea-pig or rat. We have also not detected regional differences in the potency or maximal effect of acetylcholine and carbachol or of the β-adrenoceptor agonists isoprenaline and fenoterol in either the guinea-pig or rat isolated trachea (unpublished data). Whilst the reasons for the regional change in the α-adrenoceptor population down the length of the rat trachea demonstrated in the present study are unclear, it is known the density of sympathetic innervation in guinea-pig airways diminishes down the trachea with no nerves evident in the bronchi (O'Donnell & Saar, 1973; O'Donnell *et al*., 1978). Despite these changes in innervation density, adrenergic and non-adrenergic components of smooth muscle relaxation were not significantly different in the laryngeal, cervical or thoracic segments of guinea-pig isolated tracheal tissue (Souhrada & Kivity, 1982). No such studies have been performed on the density of adrenergic innervation in the rat airways.

In the present study, there were no changes in the potencies of the α-adrenoceptor agonists, where responses could be measured, down the length of the rat trachea. Thus, regional changes observed with respect to maximal contraction may have been due to changes in the number of functional receptors and not due to changes in the affinity of the receptors for the agonist or in their ability to elicit a response.

In guinea-pig isolated trachea, there were no significant changes in α-adrenoceptor-mediated contraction, when the data were assessed across the animal age-range studied, either in terms of the contractile potencies of the agonists or the maximal contractile responses obtained. However, transient increases in the potencies of (−)-adrenaline and (−)-noradrenaline were observed during the maturation phase of growth (2–12 weeks of age) which subsided thereafter to levels seen in tissue from immature animals. This was not seen in rat trachea. The biphasic changes in adrenoceptor agonist potency observed during the maturation phase of animal growth (rather than with senescence in the guinea-pig in the present study), are consistent with similar biphasic changes in potency over the maturation phase for other spasmogens such as histamine and acetylcholine (Preuss *et al*., 1992).

Whilst Takayanagi and coworkers (1991) observed significant reductions in guinea-pig tracheal sensitivity and E_{max} to noradrenaline in mature (10–100 weeks) animals compared with values observed in tissue from immature animals (3–6 weeks), the present study has provided a more complete profile of the relationships between these parameters and animal age. The present data confirm that the potencies of both (−)-noradrenaline and (−)-adrenaline at 12 weeks were greater than the respective values at 52 and 156 weeks. However, it is also clear that the relationship between animal age and potency for these agonists is not described by a simple linear function. Importantly, the potency vs age curves are bimodal in the maturation phase of growth, after which tissue sensitivity remained essentially constant. The results of the present study thus serve to highlight the limitations in drawing conclusions from studies where only a relatively small number of age groups are examined. Furthermore, the present study highlights the importance of the maturation phase of animal growth in terms of agonist (spasmogen) potency rather than the senescence phase.

Studies confirming age-related changes of the α-adrenoceptor population by use of gene transcription have yet to be done in airway tissue. However, a study examining the expression of α-adrenoceptor genes in the superior cervical ganglion demonstrated a significant fall with maturation in the expression of the α_{1b}-adrenoceptor subtype, but no change in the expression of the α_{1c}- or α_2-adrenoceptor subtype transcripts (Vidovic & Hill, 1997). The transcription rate of rat hepatic β_2-adrenoceptors is also significantly reduced in postnatal, compared to foetal animals (Baeyens & Cornett, 1993). These studies provide evidence that changes may occur in adrenoceptor expression with respect to animal age during the maturation phase of growth.

In the absence of the β-adrenoceptor antagonist (±)-propranolol, initial relaxation responses of guinea-pig isolated tracheal tissue to both (−)-adrenaline and (−)-noradrenaline were seen. Contractile responses were observed at the higher agonist concentrations, reflecting the dominant influence of β-adrenoceptor function mediating relaxation, over α-adrenoceptor function mediating contraction. In contrast, (−)-adrenaline and (−)-noradrenaline caused contraction in rat isolated tracheal preparations in the

presence or absence of (±)-propranolol. These results are in agreement with previous findings indicating that in the absence of β-adrenoceptor blocking agents, noradrenaline exerts a strong relaxant effect with negligible contractile activity in guinea-pig trachea (Naline *et al*., 1988). Furthermore, it has been demonstrated that rat trachea and bronchi have little or no intrinsic tone (Burns & Doe, 1978). This is consistent with the failure of (±)-propranolol to alter significantly contractile responses to agonists such as adrenaline and noradrenaline which can stimulate both α- and β-adrenoceptors.

The non-selective α-adrenoceptor antagonist, phentolamine caused a significant concentration-dependent inhibition of (−)-noradrenaline-induced contraction in guinea-pig isolated tracheal tissue. The pA_2 values obtained are in line with results from previous studies in human peripheral lung (Black *et al*., 1981) and are consistent with the actions of phentolamine at α-adrenoceptors. Furthermore, the pA_2 obtained for phentolamine in the present study is in line with affinity values found for phentolamine at the α_{1b}-adrenoceptor subtype (Michel *et al*., 1995).

Indomethacin abolished contractile responses to (−)-adrenaline and (−)-noradrenaline in guinea-pig isolated trachea, indicating that contraction required the secondary release of spasmogenic cyclo-oxygenase products. This is in accord with a previous study which also demonstrated indomethacin-sensitive contraction to noradrenaline in guinea-pig isolated tracheal smooth muscle (Takayanagi *et al*., 1990). In contrast, the present study has shown that α-adrenoceptor-mediated contraction in rat isolated trachea was not affected by indomethacin and thus was not linked to the release of cyclo-oxygenase products in this species. Differential responses of guinea-pig and rat isolated trachea to the effects of indomethacin have also been demonstrated previously with respect to endothelin-mediated contraction (O'Donnell *et al*., 1990). Thus, the release of cyclo-oxygenase products is an important component of the mechanism of action of some spasmogenic agonists in guinea-pig but not rat isolated trachea. Interestingly, differential responses of similarly acting agents within the same tissue type have been demonstrated. For example, aspirin, which also inhibits cyclo-oxygenase, inhibited contractile responses of guinea-pig airway smooth muscle to the calcium agonist CGP 28392, but not to the calcium agonist Bay K8644 (Pichoff *et al*., 1993).

Whilst the influence of animal age on the activity of cyclo-oxygenase in the airways has received little attention, studies in guinea-pig whole blood have demonstrated that neither cyclo-oxygenase nor phospholipase A_2 activities were altered as a function of animal age (Spaethe *et al*., 1992). Similarly, in rat aortic smooth muscle cells, total cyclo-oxygenase activity was the same in cells from young (12 months of age) and old (24 months of age) rats (Chang *et al*., 1980). However, immunoblot analyses of intrapulmonary arteries from foetal and newborn lambs demonstrated a significant maturational increase in cyclo-oxygenase-1 protein with the cyclo-oxygenase-2 protein being undetectable. Cyclo-oxygenase-1 mRNA in whole lung also increased significantly with respect to maturation age (Brannon *et al*., 1994). In pigs, indomethacin caused a significant rightward shift in the phenylephrine cumulative concentration-effect curve of the pulmonary artery of young (5 week old) piglets, but had no effect on the responses of older (12 and 26 weeks) animals (Gustin *et al*., 1993).

In canine isolated tracheal smooth muscle, both α_1- and α_2-adrenoceptor subtypes have been demonstrated, with smooth muscle contraction predominantly mediated by α_2-adrenoceptors in this species (Barnes *et al*., 1983). Takayanagi and coworkers (1990) also concluded that α_2-adrenoceptors predominantly mediated contraction. However, the present study shows that whilst (−)-phenylephrine induced only small contractile responses of guinea-pig isolated tracheal smooth muscle, even weaker responses were obtained with the α_2-adrenoceptor agonist, clonidine. The reasons for these differences between the two investigations are unclear, but do not appear to be the result of guinea-pig strain differences. This is demonstrated by our experiments with tracheal preparations from the Hartley strain of guinea-pig, as used by Takayanagi and coworkers (1990), in which we again did not detect any significant contraction in response to clonidine. This is consistent with results from Marcelle (1996), in which α_1-adrenoceptor-mediated bronchoconstriction in response to noradrenaline and phenylephrine was demonstrated in the anaesthetized Hartley guinea-pig.

Whilst the scope of this paper did not extend to examining the subtype of α-adrenoceptor involved in mediating the contraction observed, the presence of mRNA for α_{1c}- and α_{1d}-adrenoceptors in rat lung has been demonstrated suggesting the presence of these α_1-adrenoceptor subtypes (Faure *et al*., 1994). Few, if any studies have examined the α-adrenoceptor subtypes present in the airways of the rat or guinea-pig and this is most likely due to the fact that the responses observed to α-adrenoceptor agonists in these tissues are relatively small, and in the case of the rat with significant regional differences in responsiveness to these agonists, very difficult to detect at all.

In conclusion, the present study has demonstrated α-adrenoceptor-mediated contraction in tracheal smooth muscle from the guinea-pig and the older rat. (−)-Adrenaline-induced contraction of guinea-pig tracheal smooth muscle is indomethacin-sensitive and is optimally revealed in the presence of a β-adrenoceptor antagonist. Functional responses to selective agonists suggested a more prominent role for α_1-adrenoceptors than for α_2-adrenoceptors, although contractile responses to all α-adrenoceptor agonists were generally small. Rat isolated tracheal contraction to (−)-adrenaline was indomethacin-insensitive and did not require inhibition of β-adrenoceptors for optimal expression. As in the guinea-pig, functional responses to selective agonists in the rat suggested a more prominent role for α_1-adrenoceptors than for α_2-adrenoceptors.

More significantly, we have demonstrated significant age- and region-dependent α-adrenoceptor-mediated contraction in rat isolated tracheal smooth muscle. In guinea-pig isolated tracheal smooth muscle, no regional differences were detected in α-adrenoceptor-mediated contraction, although significant age-related changes in the potencies of the α-adrenoceptor agonists were observed.

These findings are important for a complete understanding of α-adrenoceptor-mediated contraction of airway smooth muscle. Interestingly, in guinea-pigs, tracheal α-adrenoceptor function is at a peak in the age range most commonly used in biological experiments. Conversely, in rats, α-adrenoceptor-mediated contraction was insignificant in animals up to 12 weeks of age, the age group most commonly targeted by researchers. Clearly, animal age, species and airway region must be considered when studying airway α-adrenoceptor function, and its potential to contribute to airway smooth muscle tone in response to endogenous and exogenous catecholamines.

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