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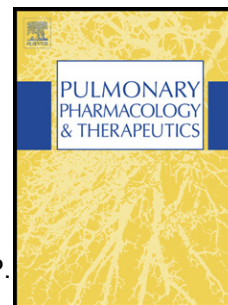
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Evaluation of the effects of the R- and S- enantiomers of salbutamol on equine isolated bronchi

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Abstract:

Background: Equine obstructive pulmonary disease, also known as heaves or recurrent airway obstruction (RAO) is a common equine pulmonary disease with some similarities to human asthma and COPD, which represents a major cause of morbidity and loss of lung performance. Salbutamol has been widely used for the treatment of human airway diseases and has usually been prepared as the racemic form of the drug. However, recently the R- enantiomer of salbutamol has been introduced into clinical practice in the treatment of asthma in humans and this has been suggested to be an improvement on the racemic form of the drug; therefore thus the S-enantiomer has been demonstrated to have adverse effects in the lung and thus using the R-enantiomer may improve the therapeutic ratio. However, little is known about the properties of the R- and S-enantiomers of salbutamol in equine airways and the present study has evaluated the relaxant effects of racemic β_2 agonists in comparison with the R- and S- enantiomers in isolated equine isolated bronchi, as well as the bronchoprotective effects of these drugs on cholinergic and histaminergic pathway.

Methods: We have studied the effects of the R- and S- enantiomers of salbutamol on bronchi isolated from RAO-affected or unaffected horses. The first study assayed the relaxant effects of R- and S- salbutamol on isolated bronchial rings contracted with carbachol or histamine at a submaximal concentration (EC70). A second study evaluated the effects of R- and S- salbutamol on semi-logarithmic cumulative concentration response curves induced by carbachol or histamine. Specific software was used to calculate statistical significance and the appropriate sigmoidal curve-fitting model.

Results: Neither enantiomers of salbutamol caused a relaxant effect on the sub-maximal plateau contractile effects of carbochol; in fact, both R- and S-salbutamol induced a slight, but significant contraction ($P \leq 0.05$) compared to the controls. In contrast, R-salbutamol induced a significant relaxation of bronchi precontracted with histamine (RAO-unaffected: $92.06\% \pm 2.00$; RAO-affected 100.20 ± 3.99 ; $P \leq 0.01$). S-salbutamol induced a weak relaxation (RAO-unaffected: $15.81\% \pm 5.65$; RAO-affected 2.36 ± 5.15) when compared to that induced by papaverine. The incubation with either R- or S-salbutamol shifted rightward ($P \leq 0.001$) the carbachol contraction curve in RAO-unaffected bronchi, but not in RAO-affected bronchi, compared to control tissues. R-salbutamol induced a reduction in E_{max} values (C: $9.07\text{gr} \pm 0.68$; R-salb.: $6.36\text{gr} \pm 0.21$; $P \leq 0.01$) in normal bronchi. On the contrary it reduced the histamine potency in RAO-affected bronchi (EC_{50} $7.10 \mu\text{M} \pm 0.35$, $P < 0.001$).

The incubation with S-salbutamol shifted leftward the histamine concentration curve in both normal bronchi (C: $7.00\mu\text{M}\pm 0.29$; S-salb.: $2.25\mu\text{M}\pm 0.19$; $P\leq 0.001$) and bronchi from RAO-affected horses (C: $2.80\mu\text{M}\pm 0.26$; S-salb.: $1.50\mu\text{M}\pm 0.80$; $P\leq 0.05$).

Conclusion: Our studies have demonstrated that S-salbutamol elicited a modest increase in contraction of equine airway smooth muscle induced by carbachol and induced a significant hyperresponsiveness to histamine. These results confirm the ability of the S-enantiomer of salbutamol to potentiate the contractile effect of certain spasmogens on airway smooth muscle. Such an adverse effect would be determined in the airways of horses with RAO and suggest that if salbutamol is to be used in the treatment of symptoms of RAO in horses, the R-enantiomer, rather than the racemic mixture should be considered.

Keywords: salbutamol, enantiomers, equine isolated bronchi, RAO.

Introduction

There is an increasing amount of evidence demonstrating an association of inhaled β_2 agonists with a rise in asthma-related deaths and life-threatening experiences, as well as an ability of β_2 -agonists to induce bronchial hyperresponsiveness when used clinically, particularly when administered as monotherapy [1]. The mechanisms contributing to the adverse effects in the airways are not known, but it has been suggested that some of these undesirable effects maybe due to the S-enantiomer as most commonly available β_2 agonists are produced as racemic mixtures of R- and S-enantiomers in equal ratios [2]. The S-enantiomer of salbutamol has been considered to be largely inert or at best to have weak β_2 -agonist activities. However, recent studies have documented that S-salbutamol can potentiate the effects of spasmogens in airway smooth muscle from both guinea-pigs and humans , with a number of clinical studies also reporting worsening of airways hyperresponsiveness in animals [3] and in subjects with asthma [4]. It has also been documented that the metabolic clearance of S-salbutamol occurs much less rapidly than R-salbutamol [5], and thus the paradoxical responses may persist after the beneficial effects of (R)-salbutamol have waned [2], particularly after chronic use of the drug. S-salbutamol has also been reported to have proinflammatory effects that could negatively affect the airways [6].

Chemically, all β -agonists are racemates, or drugs composed of two non-superimposable mirror image molecules in a 50/50 ratio [7]. These molecules are classified as R- or S- according to their molecular configuration, and as 'lev' or 'dex' based on the rotation of polarised light as it passes through the molecule.

Equine obstructive pulmonary disease, also known as heaves or recurrent airway obstruction (RAO) is a common equine pulmonary disease with certain similarities to human asthma and COPD and represents a major cause of morbidity and loss of lung performance in this species [8, 9].

Whilst β_2 -agonists are used in the treatment of RAO, little is known about the properties of the enantiomers of salbutamol in the horse. The present study has therefore evaluated effects of racemic β_2 -agonists in isolated equine bronchi in comparison with the pure R- and S-enantiomers.

Materials and Methods

Equine lungs, derived from healthy horses following humane killing in an abattoir, were obtained from six RAO-unaffected horses (3 male and 3 female; aged 2.3 ± 0.1 years; weighted kg 375 ± 54.3) and four RAO-affected horses (2 male and 1 female; aged 2.5 ± 0.2 years; weighted kg 388 ± 46.5). Lungs were rapidly transported to our laboratory in a Krebs-Henseleit solution (KH) (composition, mM: NaCl 117.5. KCl 5.60. MgSO_4 1.18. CaCl_2 2.50. NaH_2PO_4 1.28. NaHCO_3 25.00 and glucose 5.5, pregassed with O_2/CO_2 95:5% v/v. pH7.4). Segmental bronchi, typically 3-5 mm in diameter, were derived from each set of lungs and dissected free from connective tissue and cut into rings. Epithelium intact bronchial rings were transferred into organ baths containing KH-buffer (37°C), treated with indomethacin ($10\ \mu\text{M}$) in order to inhibit cyclo-oxygenase enzymes and so prevent prostaglandin formation and any intrinsic tissue activity. Tissues were continuously gassed with O_2/CO_2 95:5 v/v. Bronchial rings were connected to an isometric force displacement transducer and were allowed to equilibrate for 90 min containing modified with the KH buffer being changed every 10 min. During equilibration (2 h) optimal passive tension were determined by gentle stretching of tissue ($\cong 2\ \text{g}$). The isometric changes in tension was measured with a transducer Fort 10 WPI (Basile Instruments. Italy).

The tissue responsiveness was assessed using acetylcholine (ACh) $100\ \mu\text{M}$; when the response reached a plateau, rings were washed three times and allowed to equilibrate for a further 60 min.

Study design

Protocol 1: Relaxant effect of salbutamol enantiomers on submaximal contractions induced by carbachol or histamine.

Following equilibration of the tissues, semi-logarithmic concentration-response curves induced by carbachol (dose from 10 nM to $100\ \mu\text{M}$) or histamine (10 nM to $100\ \mu\text{M}$) were constructed in order to establish the baseline contractility of tissues and the concentration

inducing a sub-maximal contraction (70%). The bronchial rings were washed a further three times and allowed to stabilize for about 30 min following the last washing.

In order to evaluate the relaxant effects of salbutamol enantiomers, in all rings contracted with carbachol and histamine at the sub maximal concentration (EC70), a concentration semi-logarithmic curve to salbutamol enantiomers or the racemic mixture was constructed at concentrations ranging from 0.1nM to 100µM for each compound. Each concentration-response curve was obtained by the cumulative addition of R-, S- or racemic salbutamol at intervals of 5-15 min to reach a stable level of relaxation before the next addition was made. In the control groups a cumulative concentration of vehicle was added in the organ-baths and used as a time control. At the end of the experiments papaverine (500 µM) was added to the bronchial rings to determine the maximal relaxant response achievable for each isolated bronchus. Similar experiments of protocol 1 were repeated in bronchi from RAO-affected horses.

Protocol 2: Effects of salbutamol enantiomers on smooth muscle contraction induced by carbachol or histamine.

In order to evaluate the effects of salbutamol enantiomers on carbachol and histamine-induced contraction, concentration-contraction semi-logarithmic curves to carbachol or histamine (10 nM to 100 µM) were constructed from bronchi pre-incubated with R-, S- or racemic salbutamol (100 µM) for 90 min. The control bronchi were pre-incubated with vehicle which also served as a time control.

Analysis

All experiments were carried out on bronchi isolated from the lungs of six different horses (n=6). Appropriate curve-fitting to a sigmoidal model was used to calculate the effect, the maximal response, the dose inducing 50% maximal effect and the dose inducing 70% maximal effect (E , E_{max} , EC_{50} and EC_{70} respectively). The equation used was $\log(\text{agonist})$ vs. response, Variable slope, expressed as $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / \{1 + 10^{[(\log EC_{50} - X) * \text{HillSlope}]}\}$. The relaxant responses were expressed as percentage of papaverine (500 µM) induced relaxation. All values are presented as mean \pm SEM of n subject in each treatment group. Statistical significance was assessed by Student's t test or one- or two-way analysis of variance (ANOVA), with Dunnett's or Bonferroni post-tests respectively. The level of statistical significance was defined as $P \leq$

0.05. All data analyses were performed using computer software (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA).

Drugs

Acetylcholine, carbachol and histamine were dissolved in KH solution; papaverine was dissolved in distilled water; R-, S- and racemic salbutamol were dissolved in distilled water; indomethacin was dissolved in ethanol and the maximal amount of ethanol (0.02%) did not influence the response of isolated bronchi. Stock solutions (1 mM) of each of the tested substances were prepared in distilled water, diluted with KH solution and prepared fresh on each day of study. The substances were obtained from Sigma-Aldrich (St. Louis, USA), R-S- and racemic salbutamol were obtained from Stirling Products Ltd (Sydney, Australia) .

Results

Protocol 1: Relaxant effect of salbutamol enantiomers on submaximal contractions induced by carbachol or histamine

The baseline tension of bronchial tissues did not change throughout the experiments carried out with S-Salbutamol or the racemic mixture.

In the control bronchi, papaverine completely relaxed equine bronchial rings contracted with a sumaximal concentration of carbachol (CCh: $+4.37\text{gr}\pm0.42$; P: $-4.64\text{gr}\pm0.45$) or histamine (His: $+6.59\text{gr}\pm0.98$; P: $-7.93\text{gr}\pm0.85$). In contrast, salbutamol enantiomers did not show direct relaxant effects on the sub-maximal plateau contractile response induced by carbachol in either RAO-affected or RAO-unaffected bronchi. On the contrary, both enantiomers of salbutamol induced a slight but significant increase ($P\leq0.05$) of the carbachol baseline. Furthermore, racemic salbutamol showed a similar trend to the induced enantiomers (Figure 1; Table 1).

R-salbutamol induced a significant ($P<0.05$) relaxation of the histamine sub-maximal plateau contractile response (RAO-unaffected: $E_{\text{max}} 92.06\%\pm2.00$, $EC_{50} 0.55\mu\text{M}\pm0.06$; RAO-affected: $100.20\%\pm3.99$, $EC_{50} 0.40\mu\text{M}\pm0.04$) . S-salbutamol induced a weak relaxation (RAO-unaffected: $15.81\%\pm5.65$; RAO-affected: 12.36 ± 5.15) when compared to that induced by papaverine on the histamine response that was significant ($P\leq0.01$) only at the higheest concentrations used. Moreover, the racemic compound was analogous to the

R-salbutamol effect, but eliciting a smaller Emax, particularly in bronchi from RAO-affected horses (Figure 1; Table2).

In all bronchial rings the vehicle did not induce any relaxation of the bronchi.

Protocol 2: Effects salbutamol enantiomers on smooth muscle contraction induced by carbachol or histamine

Carbachol and histamine induced a concentration-dependent contraction of equine isolated bronchi; the incubation with KH did not elicit any effect on equine isolated bronchial rings (figure 2). The EC70 of carbachol and histamine are presented in Table 3. The incubation with R- or S-salbutamol shifted to the right the carbachol contraction curve compared to the controls ($P \leq 0.001$) at concentrations ranging from 0.3 μM to 100 μM ; both R- and S-salbutamol showed a significant reduction in Emax (R-salb.: $P \leq 0.001$; S-salb.: $P \leq 0.05$) with a significant increase in the EC50 (R-salb.: $P \leq 0.001$; S-salb.: $P \leq 0.05$); the trend of the carbachol induced contraction curves after the incubation with racemic mixture of salbutamol was analogous to effects elicited by the individual enantiomers (figure 2, table 3).

R-salbutamol caused a significant shift to the right of the cumulative concentration-contraction curve of histamine, with a significant reduction in Emax values (C: $9.07\text{gr} \pm 0.68$; R-salb: $6.36\text{gr} \pm 0.21$; $P \leq 0.01$) but without any significantly change on EC50 values (C: $7.00\mu\text{M} \pm 0.29$; R-salb: $7.25\mu\text{M} \pm 1.06$; $P < 0.05$). In contrast the incubation with S-salbutamol shifted leftward the histamine concentration curve when compared to the left when compared with controls, with a significant reduction of EC50 values (C: $7.00\mu\text{M} \pm 0.29$; S-salb: $2.25\mu\text{M} \pm 0.19$; $P \leq 0.001$) but without any significant change on Emax values (C: $9.07\text{gr} \pm 0.68$; S-salb: $8.12\text{gr} \pm 0.51$; $P < 0.05$). The trend of the histamine induced contraction curves after incubation with the racemic mixture of salbutamol was similar to the effects induced by the S-isomer, but with Emax values significantly different when compared to control tissues (figure 2, table 3).

The contractile response to carbachol did not change in bronchi from RAO-affected horses, whereas the contractile potency of histamine was significantly enhanced in bronchi from RAO-affected horses (EC50 $2.8\mu\text{M} \pm 0.26$), compared to tissue obtained from RAO-unaffected horses. In RAO-affected bronchi, R- and S- enantiomers of salbutamol and the racemic mixture did not alter the concentration response curve to carbachol compared to

control tissues ($P>0.05$). On the contrary, the contractile response to histamine was shifted leftwards by S-salbutamol ($EC_{50} 1.50\mu M \pm 0.80$, $P<0.05$) whereas it was shifted rightward by R-salbutamol ($EC_{50} 7.10 \mu M \pm 0.35$, $P<0.001$). In bronchi from RAO-affected horses the racemate did not change the contraction induced by histamine ($P>0.05$) (figure 2, table 3).

Discussion

Inhaled β_2 -agonists remain the first line treatment of acute symptoms of airway obstructive diseases in horses. Most commonly available β_2 -agonist preparations are racemic, i.e. they are composed of an equimolar mixture of R- and S- stereoisomers. Traditionally the S-enantiomers were considered inert or at best weak β_2 -agonists [10], but it is now recognized that the S-enantiomer can have adverse effect in the airways [2]. Thus, it has been documented in *in vitro* and *in vivo* experimental studies that S-salbutamol can be pro-inflammatory and have pro-constrictor effects in airways. These results are in agreement with clinical studies that have demonstrated that S-salbutamol can induce airway hyperresponsiveness in patients with asthma. It has been suggested that these paradoxical responses may be reduced by removing the dose of S- enantiomers [5, 11]. Besides, R-salbutamol is now commonly available for the treatment of obstructive lung disease and further R-isomers preparations of β_2 -agonist are under development in man (3)

Like human obstructive bronchial diseases, equine obstructive pulmonary disease, also known as heaves or recurrent airway obstruction (RAO), is a common equine chronic pulmonary disease, in which the prominent symptom is airway obstruction which is treated with β_2 agonists [8, 9]. Our present study shows that S-salbutamol has adverse pro-constrictory effects in equine bronchi, particularly in bronchi from RAO-affected horses.

Previous studies carried out with isolated equine trachea have demonstrated that β_2 -agonists facilitate acetylcholine release from prejunctional cholinergic nerves by the stimulation of prejunctional β_2 -receptors localized on parasympathetic nerve endings [11, 12]. Moreover, the S-enantiomers of two β_2 -agonists (salbutamol and formoterol) elicited a significant increase in acetylcholine release in the presence of M_2 receptor blockade, and suggested a potential deleterious effect of (S)- β_2 agonists in the lungs of horses with RAO [13]. To our knowledge, this is the first paper to focus on the effects of the R- and S-

enantiomers of salbutamol on isolated bronchi obtained from Either normal or RAO affected horses.

Our results are consistent with an effect of acetylcholine release, as demonstrated by Zhang and co-workers [12-14]. Furthermore, it has also been postulated that S-salbutamol has a possible agonistic effect on M_3 muscarinic receptors postjunctionally, via a mechanism that involves an increase of intracellular calcium in airway smooth muscle [13, 14]. According with this hypothesis, and considering that the affinity of S-isomer for β -adrenoceptors is >100 fold lower than R-isomer, Mitra and coworkers suggested that S-salbutamol could work as an agonist for muscarinic receptors cross-reacting with M_3 receptors, acting also as a calcium agonist [14]. Moreover, several studies have shown that an exaggerated cholinergic stimulation of airway smooth muscle causes a reduction in the ability of β_2 -agonists to relax airway smooth muscle [16, 17]. These results could suggest that both enantiomers of salbutamol could have deleterious effects in equine airways, particularly in RAO-affected horses. However, our results have demonstrated that R-salbutamol functionally antagonized the carbachol concentration contraction curve in normal bronchi, but not in bronchi obtained from horses with RAO. Furthermore, R-salbutamol postjunctionally inhibited the smooth muscle tone induced by both histamine and carbachol in a significant manner in both normal and RAO bronchi. In addition, on equine carbachol precontracted tracheal strips, we have provided evidence that S-salbutamol had a modest, but significant, bronchoconstrictor effect [13, 18]. These data may support the deleterious role of S-salbutamol, particularly in RAO-affected horses, in which the β_2 -adrenergic receptor- G_s -protein-adenylate cyclase system is significantly impaired in membranes of peripheral lung and bronchi, when compared with the segments from control animals [19].

In contrast with the observations on carbachol precontracted equine bronchi, the results of our study demonstrated that R-salbutamol relaxed equine isolated airways contracted with histamine. Moreover, the cumulative concentration-contraction curve to histamine was significantly shifted rightward with a significant increase of its EC₅₀ when both normal and RAO equine bronchi were incubated with R-salbutamol, compared to controls tissues.

Our findings are in line with results obtained in bovine tracheal smooth muscle, in which the contraction induced by muscarinic agonists was relatively resistant to relaxation by β_2 -agonists, compared to the contraction induced by histamine [17]. In fact, this spasmogen

induced a less marked calcium mobilization and influx when compared to that induced by methacholine [16], which is less susceptible to elevations of c-AMP [20].

As expected, we observed a less marked relaxant effect of S-salbutamol, that was significant only at the highest concentration and certainly less effective than papaverine. In addition, we found a significant leftward shift of the histamine cumulative concentration contraction curve, with an increase of histamine potency, when normal airways or bronchi from RAO-affected horses were incubated with S-salbutamol, compared to control tissues. In line with these results, it has been documented that detrimental effects of the racemic mixture of β_2 -agonists are strictly related to the S-isomers [2, 6, 21, 22]. Although, S-salbutamol induced a weak relaxation of equine isolated bronchi pre-contracted by histamine, it induced an increase in airway smooth muscle responsiveness to the cumulative dose response to this spasmogen in both normal and RAO bronchi. In agreement with these findings, racemic salbutamol induced an exaggerated contractile response to histamine in airway tissues obtained from sensitized guinea pigs [21]. It has been speculated that the pro-inflammatory and pro-constrictory activity of S-isomer, are mediated by the activation of transcriptional nuclear factor κ B (NF- κ B) as has been demonstrated in human isolated airways[23]. These findings were also confirmed in other animal models of bronchial hyperresponsiveness. Thus it has been reported that S-salbutamol potentiates the pro-constrictory activity of histamine and IL-4 in sensitized guinea pigs [24], by stimulating the production of IL-2 and IL-13 in T-cells [25]. Over all, these cytokines shift the coupling of the β_2 -adrenergic receptors to pro-constrictory G proteins such as $G_{i\alpha-1}$ protein [19]. Considering all these data, it is possible to hypothesize an analogous pro-inflammatory and pro-constrictory activity of S-salbutamol also in equine airways after only 90 min. of incubation, enhancing the potency of histamine, as has been demonstrated in human bronchi after passive sensitization [26].

In conclusion, our studies demonstrated that S-salbutamol elicited a weak contraction of equine airway smooth muscle, but induced a significant hyperresponsiveness to histamine that could be detrimental particularly in RAO-affected horses [27-30]. Our results suggest that it may be prudent to administrate the R-isomer alone in RAO horses to avoid detrimental effect of S-isomer.

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Table 1. Relaxant effects of R, S and racemic mixture of salbutamol on equine isolated bronchi (normal and RAO-affected horses) contracted with carbachol (negative values mean a contraction). All values are mean \pm SEM of six (normal bronchi) and four (bronchi from RAO horses) samples. * $P \leq 0.05$ vs baseline; ° $P \leq 0.05$ vs normal bronchi; nd: not detectable.

		Treatment			Papaverine relaxation Emax (g)	SubMax contraction CCh (g)
		Emax (%)	Emax (g)	EC50 (μ M)		
Normal bronchi	R salbutamol	*-8.98 \pm 0.71	*-0.35 \pm 0.03	nd	3.97 \pm 0.54	3.94 \pm 0.55
	S salbutamol	*-12.34 \pm 0.50	*-0.63 \pm 0.02	nd	5.08 \pm 0.52	5.12 \pm 0.54
	Racemic	*-9.98 \pm 0.84	*-0.45 \pm 0.03	nd	4.53 \pm 0.39	4.69 \pm 0.41
Bronchi from RAO horses	R salbutamol	-9.90 \pm 0.81	-0.43 \pm 0.02	nd	4.33 \pm 0.42	4.02 \pm 0.46
	S salbutamol	°-17.71 \pm 0.89	°-0.46 \pm 0.03	nd	4.86 \pm 0.71	4.68 \pm 0.45
	Racemic	-12.25 \pm 0.74	-0.51 \pm 0.03	nd	4.51 \pm 0.58	4.24 \pm 0.48

Table 2 - Relaxant effects of R- and S- salbutamol on equine isolated bronchi (normal and RAO-affected horses) contracted with histamine. All values are mean \pm SEM of six (normal bronchi) and four (bronchi from RAO horses) samples. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$ vs baseline; ° $P \leq 0.05$ and ∞ $P \leq 0.01$ vs normal bronchi; nd: not detectable.

		Treatment			Papaverine relaxation Emax (g)	SubMax contraction His (g)
		Emax (%)	Emax (g)	EC50 (μ M)		
Normal bronchi	R salbutamol	***92.06 \pm 2.00	***8.55 \pm 0.18	***0.55 \pm 0.06	9.29 \pm 0.31	7.56 \pm 1.00
	S salbutamol	**15.81 \pm 5.65	**1.16 \pm 0.41	nd	7.40 \pm 0.97	5.07 \pm 1.06
	Racemic	***81.12 \pm 3.67	***7.13 \pm 0.74	***0.30 \pm 0.04	8.79 \pm 0.92	6.68 \pm 0.83
Bronchi from RAO horses	R salbutamol	°100.20 \pm 3.99	°9.43 \pm 0.29	°0.40 \pm 0.04	9.42 \pm 0.29	8.98 \pm 0.86
	S salbutamol	12.36 \pm 5.15	1.03 \pm 0.33	nd	8.30 \pm 0.85	8.02 \pm 1.12
	Racemic	°64.08 \pm 3.55	°5.54 \pm 0.56	°0.20 \pm 0.03	8.65 \pm 0.98	8.50 \pm 0.98

Table 3 - Effect of R, S and racemic mixture of salbutamol on equine isolated bronchi concentration-dependent curve to carbachol (CCh) and histamine (His). All values are mean \pm SEM of six samples. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ vs controls; # $P \leq 0.001$ vs normal bronchi.

		CCDR CCh			CCDR His		
		E _{max} (g)	EC ₅₀ (μ M)	EC ₇₀ (μ M)	E _{max} (g)	EC ₅₀ (μ M)	EC ₇₀ (μ M)
Normal bronchi	Control	6.97 \pm 0.15	0.80 \pm 0.03	1.63 \pm 0.05	9.07 \pm 0.68	7.00 \pm 0.29	13.8 \pm 0.97
	R salb. incub.	§5.32 \pm 0.31	§2.50 \pm 0.24		**6.36 \pm 0.21	7.25 \pm 1.06	
	S salb. incub.	*5.67 \pm 0.44	*2.35 \pm 0.69		8.12 \pm 0.51	***2.25 \pm 0.19	
	Racemic incubation	**5.42 \pm 0.39	**2.44 \pm 0.52		*7.31 \pm 0.40	***2.00 \pm 0.25	
Bronchi from RAO horses	Control	6.96 \pm 0.18	1.00 \pm 0.16	1.55 \pm 0.09	8.34 \pm 0.28	#2.80 \pm 0.26	#5.50 \pm 0.45
	R salb. incub.	7.07 \pm 0.26	1.30 \pm 0.12		8.37 \pm 0.27	***7.10 \pm 0.35	
	S salb. incub.	6.84 \pm 0.21	0.90 \pm 0.08		7.91 \pm 0.34	*1.50 \pm 0.80	
	Racemic incubation	6.89 \pm 0.31	1.20 \pm 0.11		7.78 \pm 0.30	2.50 \pm 0.34	

Figure 1 - Relaxant effects of R, S and racemic mixture of salbutamol on isolated bronchi from RAO-unaffected (A) and RAO-affected (B) horses, pre-contracted with submaximal dose of carbachol (CCh) or histamine (His). All values are mean \pm SEM of six (A) and four (B) samples. * $P \leq 0.05$, # $P \leq 0.01$, § $P \leq 0.001$, vs CCh or His baseline plateau; @ $P \leq 0.05$ vs bronchi from normal horses.

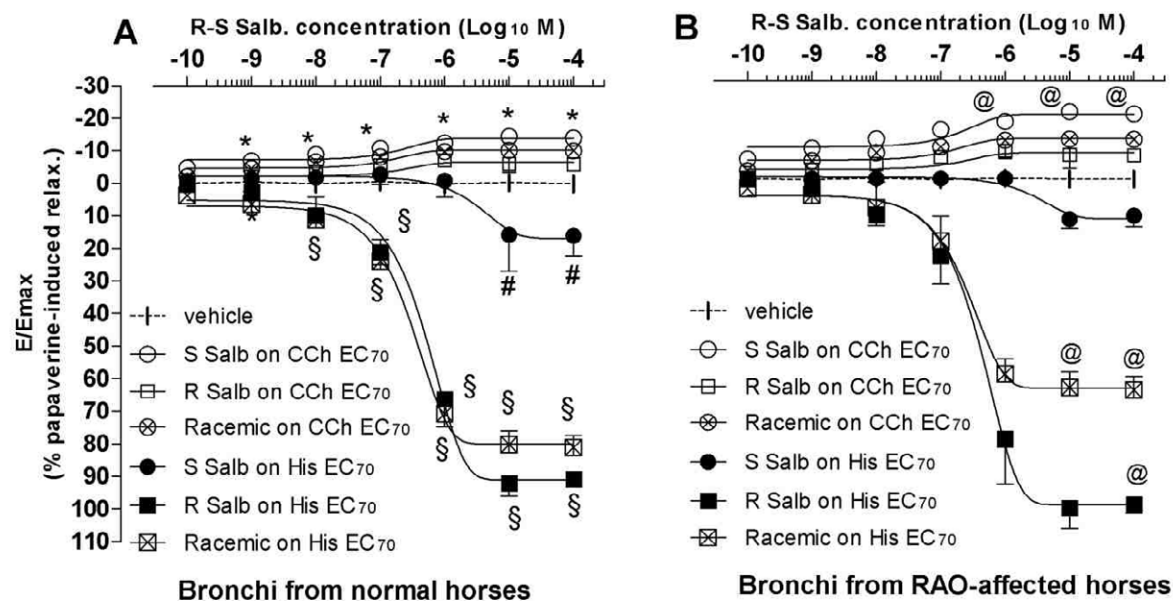


Figure 2 - Effects of R, S and racemic mixture of salbutamol on the dose response curve to carbachol (CCh, A and B) and histamine (His, C and D) in bronchi from RAO-unaffected (A and C) and RAO-affected (B and D) horses. All values are mean \pm SEM of at the six (A and C) and four (B and D) samples. * $P \leq 0.05$, # $P \leq 0.01$, § $P \leq 0.001$ vs control.

