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HETEROGENEITY OF TRACHEOBRONCHIAL LYMPHATIC SMOOTH MUSCLE RESPONSES TO HISTAMINE AND 5-HYDROXYTRYPTAMINE

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ABSTRACT

We assessed the responsiveness of tracheobronchial lymphatic smooth muscle to mediators of inflammation to determine whether homogeneous responses to histamine and 5-hydroxytryptamine (5-HT) are demonstrated among species typically used in studies of lymph vessels. Fresh porcine and bovine tracheobronchial lymph vessels were suspended from force-displacement transducers in baths containing oxygenated Krebs solution. Concentration-response curves were generated by cumulative addition of histamine (10^{-7} to 10^{-3}M) or 5-HT (10^{-7} to $3x10^{-4}$M). Active tension (AT) was expressed in milligrams and as a percentage of initial vessel ring response to 65mM KCl. Histamine elicited concentration-dependent contraction, yielding maximum AT in porcine rings of 1116±127mg (n=39; 129.1±10.5% of KCl response) and in bovine rings of 733±106mg (n=20; 65.8±12.9%; P=0.0005 for percent responses). PD_2 values (negative log_{10} of the concentration at half-maximum effect) were 4.49±0.08 and 4.82±0.08; (P=0.0034). 5-HT elicited concentration-dependent contraction, yielding maximum AT of 560±50mg in porcine rings (n=15; 97.2±9.7%) and 2892±454mg in bovine rings (n=27; 159.0±29%; P<0.0001 for percent responses). PD_2 values were 6.25±0.05 and 5.28±0.04 (P<0.0001). The data demonstrate a role for inflammatory mediators in the modulation of tracheobronchial lymphatic smooth muscle tone that is species- and mediator-specific, and support the potential for paracrine regulation of tracheobronchial lymph flow.

Lymph vessels possess intrinsic smooth muscle activity that is an important determinant of lymph flow (1,2). Modification of this activity by mediators of inflammation (3-10) may influence lymph flow and contribute to the pathophysiology of edema formation and resolution. The effects of mediators of inflammation on vascular smooth muscle are variable, differing significantly among both mediators and species (11-13). Whether similar heterogeneous effects exist in lymph vessels is unknown.

We sought to assess the responsiveness of tracheobronchial lymphatic smooth muscle to mediators of inflammation, to determine whether such responses were uniform across species typically used in studies of lymph vessels. We compared the effects of histamine and 5-hydroxytryptamine (5-HT) on isolated porcine and bovine tracheobronchial lymphatic vessel rings and demonstrated quantitatively different responses between mediators within species and to each mediator

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between species. The data are consistent with a role for inflammatory mediators in the regulation of tracheobronchial lymphatic smooth muscle tone that is species- and mediator-specific, and support the potential for paracrine regulation of tracheobronchial lymph flow.

MATERIALS AND METHODS

Tissue Preparation

Blocks of mediastinal tissue from freshly slaughtered pigs (125-225kg; male or female) and cattle (150-250kg; male or female) were immersed in warm saline for transport and prepared within 30 min of harvest as previously described (14-16). A 1% solution of Evans blue in saline was injected into lymph nodes at the tracheobronchial junction, and the tissue blocks were allowed to incubate at 37°C for 30 min to permit staining of efferent lymph collectors. Vessels measuring 2-5mm in diameter were ligated downstream from the direction of flow and dissected from surrounding tissue under a binocular microscope using microscissors. The vessels were cut into 5mm wide rings, each of which was suspended in a water-jacketed bath (initial volume 8ml) containing buffered Krebs solution (NaCl 118mM; $NaHCO_3$ 24mM; KCl 4.7mM; KH_2PO_4 1.2mM; $CaCl_2$ 1.6mM; $MgCl_2$ 0.4mM; dextrose 5.5mM; pH 7.4) at 37°C gassed with 95% O_2 and 5% CO_2. Each vessel ring was mounted onto two rigid stainless steel wires. One wire was attached to a rigidly held glass rod within the bath. The other was fixed using a loop of 0000 braided silk ligature to a Grass FT.03 force-displacement transducer that was rack and pinion mounted using an X-Y micromanipulator (Stoelting Co.) to permit stretching the tissue preparations to desired lengths. Isometric smooth muscle tension was continuously recorded on a Grass polygraph (Model 7).

Following a 1 hr equilibration period, resting tension was set at 500mg. Each ring was exposed initially to 65mM KCl-substituted perfusate to assess tissue responsiveness, then was rinsed several times with fresh perfusate until active tone returned to baseline, and subsequently was exposed to cumulative additions of contractile agonists (see below). Following completion of the experiments, vessel rings were gently blotted and weighed.

Assessment of Responses to Histamine and 5-Hydroxytryptamine

Tension produced by the vessel rings was measured in response to cumulative addition of either histamine (10^{-7} to 10^{-3}M) or 5-HT (10^{-7} to $3x10^{-4}$M for bovine rings; 10^{-7} to 10^{-4}M for porcine rings). Active tension was calculated by subtracting resting tension from total tension, and was expressed both in milligrams and as a percentage of initial individual vessel ring active tension developed in response to 65mM KCl-substituted perfusate. Cumulative concentration-response curves were generated, and both the concentration at half maximum effect (EC_{50}) and the negative $\log_{10}$ of EC_{50}, or PD_2, were calculated for both histamine and 5-HT for each tissue.

Drug Preparation

The drugs used were histamine dihydrochloride and 5-hydroxytryptamine creatinine sulfate complex (Sigma). Both were prepared in Krebs solution at dilutions appropriate to yield final organ bath concentrations described in the text.

Data Analysis

All data were expressed as mean ± SEM. Means of active tensions and PD_2 values generated in response to either histamine or 5-HT were compared between species using unpaired t tests. Statistical significance was declared when $P<0.05$.

RESULTS

Data from 54 porcine and 47 bovine

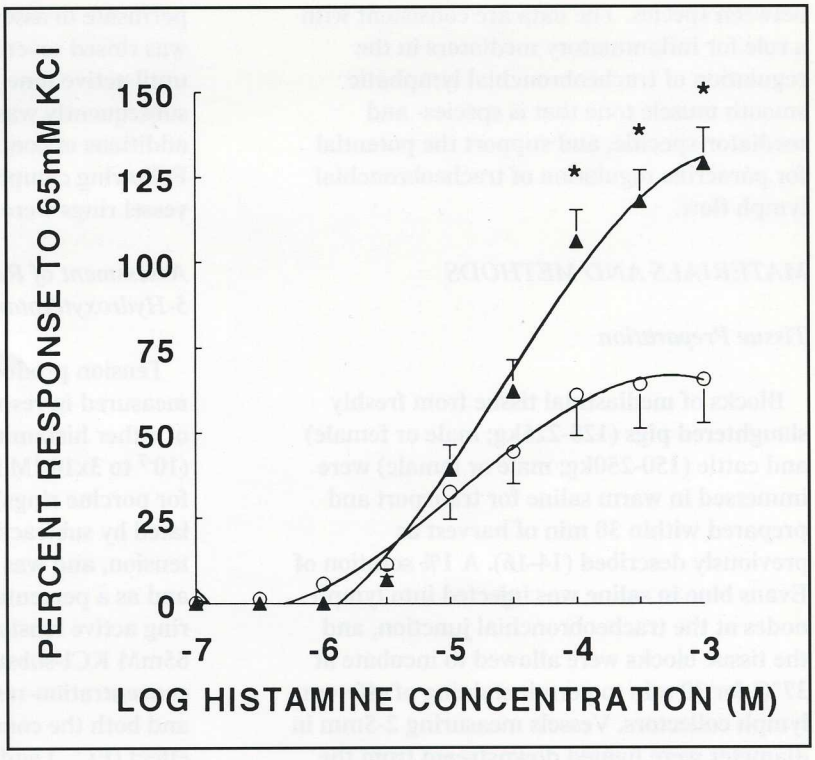

*Fig. 1. Tracheobronchial lymphatic smooth muscle responses to histamine. Active tension is expressed as a percentage of initial response to 65mM KCl versus log histamine concentration for bovine (circles) and porcine (triangles) vessel rings. Error bars represent SEM. *=P<0.05 versus bovine response at that concentration.*

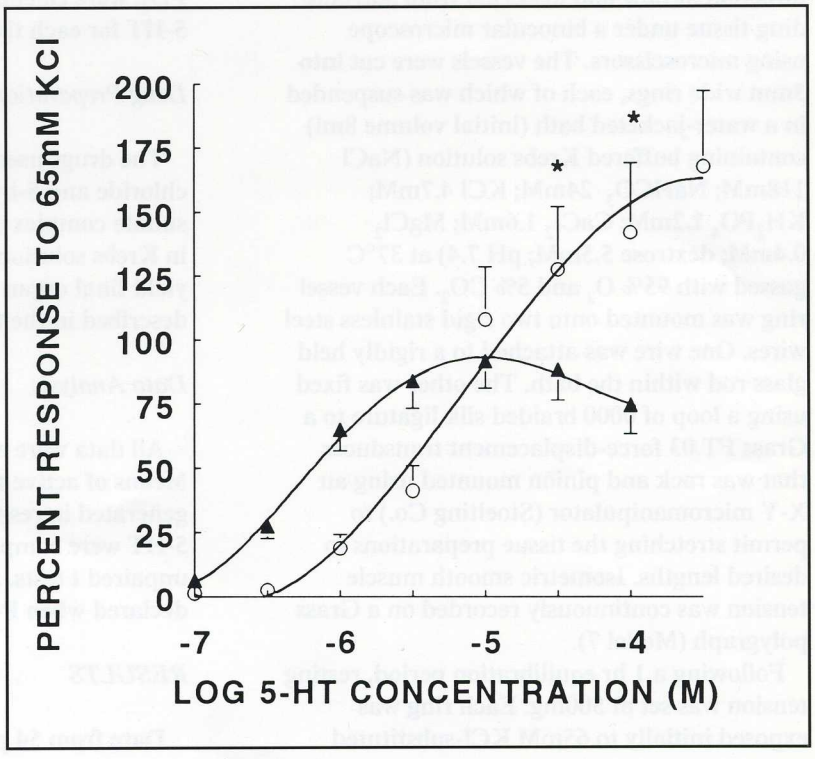

*Fig. 2. Tracheobronchial lymphatic smooth muscle responses to 5-hydroxytryptamine. Active tension is expressed as a percentage of initial response to 65mM KCl versus log 5-hydroxytryptamine concentration for bovine (circles) and porcine (triangles) vessel rings. Error bars represent SEM. *=P<0.05 versus porcine response at that concentration.*

tracheobronchial lymph vessel rings from 10 pigs and 18 cows were analyzed. The mean porcine vessel ring weight was 8.91±0.24mg, compared to a mean bovine vessel ring weight of 4.95±0.34mg (P<0.0001). Active tensions developed in response to 65mM KCl substituted perfusate were 904±87 and 1890±191mg for porcine and bovine rings, respectively (P<0.0001).

Histamine elicited concentration-dependent contractile responses in all vessel ring segments studied *(Fig. 1)*. Maximum active tension (AT_{max}) was developed in both porcine and bovine rings at a concentration of 10^{-3}M. In 39 porcine vessel rings, mean AT_{max} was 1116±127mg, compared to 733±106mg in 20 bovine vessel rings (P=0.024). Expressed as a percentage of initial vessel ring response to 65mM KCl, AT_{max} was 129.1±10.5% and 65.8±12.9% in porcine and bovine rings, respectively (P=0.0005). The EC_{50} for histamine was $3.3x10^{-5}$M in porcine rings and $1.5x10^{-5}$M in bovine rings. Corresponding PD_2 values were 4.49±0.08 and 4.82±0.08, respectively (P=0.0034).

5-Hydroxytryptamine elicited concentration-dependent contractile responses in all vessel ring segments studied *(Fig. 2)*. AT_{max} for porcine vessel rings was developed at a 5-HT concentration of 10^{-5}M, while in bovine rings it was developed at $3x10^{-4}$M. In 15 porcine vessel rings, the AT_{max} was 560±50mg compared to 2892_454mg in 27 bovine vessel rings (P<0.0001). Expressed as a percentage of initial vessel ring response to 65mM KCl, AT_{max} was 97.2±9.7% and 159±29% for porcine and bovine rings, respectively (P<0.0001). The EC_{50} for 5-HT in porcine vessel rings was $5.7x10^{-7}$M, while that in bovine rings was $5.3x10^{-6}$M. Corresponding PD_2 values were 6.25±0.05 and 5.28±0.04 for porcine and bovine rings, respectively (P<0.0001).

DISCUSSION

Lymph vessels are important determinants of accumulation and resolution of excess interstitial fluid. The function of lymph vessels is modulated by a number of factors, including activation of smooth muscle receptors by circulating or locally released mediators of inflammation and other substances (3-10,17-19), neural mechanisms (20), endothelium-derived factors such as nitric oxide and endothelin-1 (14,21,22), and complex preload and afterload determinants of lymph vessel pumping activity (1,23-26). Most experimental data regarding the responses of lymphatic smooth muscle to these modulators are derived from studies on mesenteric lymph vessels, and may not be representative of lymphatic vascular activity in general.

Previously, we reported that tracheobronchial lymph vessels demonstrate length-tension characteristics typical of muscular vessels, suggesting the potential for autoregulation of tracheobronchial lymph flow (16). Others have suggested that morphological as well as functional differences may exist in lymph vessels both among species and among different anatomic regions within a species (27). Differences among species previously have been demonstrated among regional blood vessels, particularly in their responses to mediators of inflammation (28,29). In the present study, we examined whether a similar heterogeneity of responsiveness exists in lymph vessels. The identification of variations in either the quality or magnitude of responses among lymph vessels may influence concepts regarding the function of lymphatics in specific organ beds.

We assessed the responses of tracheobronchial lymph vessel rings to histamine and 5-HT. We selected bovine and porcine tissues for use because they have been more extensively studied than other lymph vessels, and the model we employed in these experiments was similar to those previously described by us and others (3,4,14). Our previous work demonstrated poor correlation between vessel ring diameter or vessel ring weight and maximum active tension generated in response to agonist (16), possibly due to variations among vessels in both smooth

muscle cross-sectional area and orientation. Therefore, neither vessel ring weight nor diameter was suitable for use in establishing appropriate vessel ring resting tensions. We elected not to perform repeated contractile agonist administration to determine optimal resting tension to avoid inducing uncontrolled degrees of tissue fatigue that might confound our results. Our previous studies on length-tension relationships in bovine (16) and porcine (unpublished data) tracheobronchial lymph vessels demonstrated a gradual ascending limb and a broad peak for maximal active tension as optimal vessel ring length was achieved. We selected a resting tension of 500mg for vessel rings in the present experiments to coincide with points on the ascending limb or plateau of the length-tension curves for these tissues, a value that approaches and does not exceed optimal resting tension for tracheobronchial lymph vessel rings.

Histamine and 5-hydroxytryptamine were selected for use in these experiments for several reasons. Previous studies have demonstrated that *in vitro* responses of vascular smooth muscle to these substances are heterogeneous (28,29). Some blood vessels, including rabbit aorta and guinea pig pulmonary artery, exhibit variable contractile responses and agonist sensitivity to histamine, while in most other models relaxant responses are observed in response to histamine. 5-HT typically elicits vascular smooth muscle contraction, although the contraction amplitudes and effector concentrations are variable. The effects of both histamine and 5-HT on lymph vessels have been extensively examined and are among the best charac-terized mesenteric lymphatic smooth muscle agonists (3,8,10), and thus are appropriate for use in the comparative study of tracheobronchial lymphatics.

Both mediators elicited concentration-dependent contractile responses in all rings examined. Effector concentrations of histamine and 5-HT and the amplitudes of contractions generated in response to them were similar to those reported for bovine mesenteric lymph vessels and canine thoracic duct (3,10). The mean effector concentrations for each mediator differed significantly between species, with a greater difference observed for 5-HT *(Fig. 2)* compared to histamine *(Fig. 1)*. The amplitude of the response of bovine rings to 5-HT was more than twice that for histamine, while in porcine rings the amplitude of histamine contractile responses was 33% greater than that for 5-HT. The data demonstrate heterogeneous responses to mediators of inflammation in the lymphatic vascular system.

The mechanism responsible for hetero-geneity of lymph vessel responses may be as complex as that for blood vessel responses to these vasoactive substances. The amplitude of histamine-induced contractile and relaxant responses of blood vessels depends on both the species and the anatomic origin of the vascular tissue. The predominant response in the systemic and coronary arterial systems at low concentrations of histamine is one of relaxation, mediated primarily by endothelial H_1-histamine receptor-linked release of endothelium-derived relaxing factor and by smooth muscle H_2-receptors (13,30). Greater histamine concentrations or removal of the endothelium elicit concentration-dependent contractions mediated by smooth muscle H_1-receptors. Data regarding heterogeneity of 5-HT responses in blood vessels are similarly complex (11,12,30).

Data from the present experiments demonstrate that histamine and 5-HT have predominantly contractile effects on tracheobronchial lymph vessels. These effects are most likely mediated by H_1 and 5-HT_2 receptors located on lymphatic smooth muscle (30). The observed variability of the response amplitudes may be secondary to differences in smooth muscle histaminergic and serotonergic receptor concentrations and subtypes. In addition, this variability may be influenced by stimulated release of endothelium-derived relaxing factor, which is known to be elaborated by tracheobronchial lymph vessels

(14). However, because we initiated these studies to determine species and inflammatory mediator characteristics of normal tracheobronchial lymph vessel responses, specific receptor subtype analysis and the effect of endothelium-derived relaxing factor were not assessed.

We demonstrate 1) inflammatory mediators regulate tracheobronchial lymphatic smooth muscle tone and 2) the regulation of tracheobronchial lymphatic smooth muscle tone is mediator-specific. These data suggest that inflammatory mediators have a paracrine function in the pathophysiology of pulmonary lymph flow. Modulation of tracheobronchial lymph flow and its influence on the development and resolution of pulmonary edema are complex, involving not only circulating and locally released substances but neural mechanisms, endothelium-derived factors, and pressure-flow relationships. The relative importance of mediators of inflammation in the overall regulation of tracheobronchial lymph flow has yet to be determined.

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