Effect of histamine on lung contractile elements in growing cattle

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Objective—To determine the effect of histamine on the contractile elements of the respiratory tract in neonatal calves and young adult cattle.

Sample Population—Samples of trachealis muscle, bronchi, and intrapulmonary arteries and veins dissected from the respiratory tracts of healthy bovids (2 to 8 days and 16 to 20 months old).

Procedure—Histamine cumulative concentration-effect curves (10^{-8} to 10^{-3}M) were constructed in duplicate smooth muscle samples mounted in organ baths. Contractile responses to histamine were compared with reference contractions elicited by methacholine (10^{-5}M) for airways or KCl (127mM) for vessels.

Results—In young adult cattle, trachealis muscle had a substantial contractile response to histamine (84% of methacholine-induced contraction), whereas bronchi reacted slightly (15 and 20% for large and small bronchi, respectively). Although contractile responses to KCl were comparable in arteries and veins, histamine-induced contractions were greater for intrapulmonary veins than for arteries (202 vs 48% of KCl-induced contraction). In neonatal calves, histamine-induced contraction of veins also exceeded that of arteries (230 vs 54% of KCl-induced contraction); however, unlike in young adult cattle, histamine produced notable contraction of large and small bronchi (48 and 60% of methacholine-induced contraction, respectively).

Conclusions and Clinical Relevance—Compared with intrapulmonary arteries, intrapulmonary veins have greater contractile responses to histamine in neonatal and young adult cattle. Data suggest loss of histamine responsiveness in bronchial smooth muscle as neonatal calves grow to young adults. Venodilation may be useful in treatment of lung edema in cattle. (*Am J Vet Res* 2003;64:819–822)

The mucosa of the bronchial tree has high concentrations of mast cells, which can degranulate in response to immunologic or nonimmunologic stimuli.[1] The main bioactive substance released by mast cells during degranulation is histamine, which induces tracheobronchial spasms, pulmonary arterial vasoconstriction, and an increase in capillary permeability. Nevertheless, there are some exceptions to this; the tracheal smooth muscles of cats[2] and rhesus monkeys[3] and those of the bronchi in sheep[4] relax in response to histamine. Furthermore, whereas histamine clearly acts as a pulmonary arterial vasoconstrictor in rabbits[5] and humans,[6] it appears to have a venoconstricting effect in sheep,[7] dogs,[5,8] and guinea pigs.[9]

Cattle have many more pulmonary mast cells than do other species,[10] which supports the theory that histamine has a much more important role in diseases of the respiratory tract, including infectious diseases, in that species.[11] However, studies[12,13] of the reactivity to histamine of the smooth tracheobronchial and vascular muscles in cattle have provided contradictory data. In vivo, IV infusion of histamine in neonatal calves increases pulmonary resistance and static pressure-volume hysteresis and decreases dynamic compliance but fails to change static compliance.[12] These changes are compatible with constriction of both large and small airways without a change in lung elasticity. However, IV administration of histamine to middle-aged calves produces tachypnea, systemic hypotension, and pulmonary hypertension and is accompanied by a very severe pulmonary oedema.[13] These conflicting observations may result from changes in the responses of airways to histamine that develop with age; the purpose of the study reported here was to identify in vitro the effect of histamine on the various contractile elements of the respiratory system in growing calves.

Materials and Methods

Animals—Healthy neonatal calves (2 to 8 days old) and young adults (16 to 20 months old) of the Belgian Blue breed were included in the study. The tissues from the young adult cattle were obtained from a local abattoir. The neonatal calves originated from the University of Liège herd. Animals were killed by stunning with a captive bolt, followed immediately by exsanguination. For use in our study, the upper respiratory tract (larynx, trachea, and stem and lobar bronchi) of a calf had to be free of blood, mucus, fibrin, pus, ulcers, or parietal oedema; the lungs had to appear grossly normal, with no evidence of hemorrhage, consolidation, gas trapping, or emphysema. Finally, a histologic examination was carried out retrospectively on the bronchi and vessels used to confirm the absence of inflammatory lesions. The study was approved by the university's animal care and use committee.

Sample collection—Within a short period after slaughter (< 30 minutes), the trachea or apical lobe of the right lung was removed and immersed in Krebs-Henseleit solution (composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.6; KH_2PO_4, 1.16; $NaHCO_3$, 25; $MgSO_4$, 1.2; and glucose, 11.6) and kept at 4°C. Whether obtained from the trachea, bronchi, or vessels, each sample was prepared in duplicate each time and removed from a standardized anatomic location.

In young adults, 2 small adjacent rectangle-shaped samples (10 × 3 mm) of **trachealis muscle (T)** were dissected from the distal third of the trachea. Particular care was taken to excise samples of identical size to minimize variability in biological responses. In calves and young adults, bronchial

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samples were taken either from the **lobar bronchus** (**large-B**; external diameter, 4 and 10 mm in neonatal calves and young adults, respectively) or from a **small bronchus** (**small-B**; external diameter, 1 to < 4 mm in neonatal calves and young adults, respectively). For each sampling, 2 adjacent rings (3 mm in length) were prepared. Two adjacent rings (3 mm in length) were obtained from an **intrapulmonary artery (IPA)** and **intrapulmonary vein (IPV)**; the external diameter of these rings was 1 to 2 mm and 3 to 4 mm, respectively. Surrounding connective tissue was removed from all samples, and particular care was taken to maintain the integrity of the epithelium and endothelium.

Tissue specimens were placed in 10-mL water-jacketed organ baths[a] containing Krebs-Henseleit solution that was maintained at 37°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2. The isometric contraction was measured by use of a force-displacement transducer[b] coupled with a tension amplifier and recorded on a chart recorder.

Experimental design—During an initial 90-minute period of equilibration, the Krebs-Henseleit solution was replaced every 10 minutes. To offset the spontaneous changes in resting tone, airway and vascular smooth muscle were maintained at a resting force of 2 × *g* and 1.5 × *g*, respectively. After equilibration, methacholine[c] (10^{-5}M) or KCl[d] (127mM) was added to the organ baths to determine the reference contractile response of the airway or vascular smooth muscle, respectively. The preparations were washed until the baseline resting tension was restored (60 minutes).

The isometric contractile response to the cumulative addition of histamine[c] was recorded in tissues from 9 (IPA and IPV), 8 (T), and 6 (large-B and small-B) young adults and from 6 neonatal calves (IPA, IPV, large-B, and small-B). Histamine was added in log increments, from 10^{-8} to 10^{-3}M. After each maximum histamine contraction, 3 successive washing procedures were performed, followed by a washing procedure every 10 minutes for 50 minutes to restore the initial resting tension. At this point, the response to the methacholine or KCl was again measured and compared with the reference response. If the response was changed from the reference response by more than 20%, the results of the experiment were rejected. The drugs were prepared on the day of the experiment in physiologic saline (0.9% NaCl) solution; the concentrations reported are expressed as the final concentrations in the baths.

Data analyses—Contractile responses were expressed as absolute changes in tension (*g*), which were then converted to a percentage of the reference contraction induced by methacholine or KCl. Average dose-response curves for each animal were constructed with the results yielded by 2 (adjacent) samples that were studied simultaneously. The efficacy of histamine was expressed as the **maximal contractile response elicited** (E_{max}). The potency was calculated as the pD_2 value, which is equal to –log EC_{50}, where EC_{50} is the molar concentration of histamine required to produce 50% of maximal contractile response elicited.

Statistical analyses—Data collected for each animal and, subsequently, per group (T, large-B, small-B, or IPA and IPV) were expressed as mean ± SEM and compared with a Student *t* test. Differences in E_{max} values (%) between groups (large-B vs small-B or IPA vs IPV) and between ages were assessed by ANOVA and followed by a *t* test. Values of $P < 0.05$ were considered significant.

Results

Log concentration-response curves to histamine in T, large-B, and small-B samples (**Fig 1**) and in IPA and IPV samples (**Fig 2**) were obtained. In T, large-B, and small-B samples from young adult cattle, maximal contraction induced by histamine was 83.6 ± 5.4, 15.3 ± 5.1, and 20.0 ± 4.8% of the reference methacholine-induced contraction, respectively. Responses of the large-B and small-B specimens were not significantly different ($P > 0.05$); however, compared with contraction of trachealis muscle, bronchial smooth muscle contraction was dramatically less ($P < 0.001$). Sensitivity of intrapulmonary airways to histamine was weak, and the threshold concentrations were > 10^{-6}M. The pD_2 values for intrapulmonary airways were not reported, because the extremely weak response of the large-B and small-B preparations did not allow calculations to be made reliably. For T samples, the pD_2 value was 4.89 ± 0.12. Contrary to data collected from young adult cattle, histamine produced a significant contraction of large-B and small-B samples from neonates (**Table 1**); maximal contraction was 47.8 ± 9.3 and 59.9 ± 5.4% of methacholine-induced contraction for large-B and small-B tissues, respectively.

Of the vascular smooth muscle responses observed in young adult cattle, KCl-induced contraction in IPAs (5.97 ± 0.65 × *g*) was not significantly different from

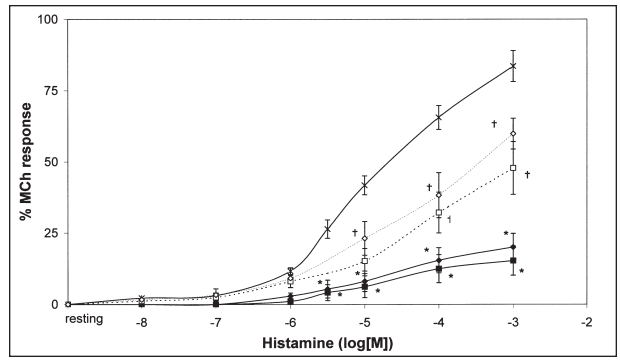

Figure 1—Cumulative dose-response curves to histamine in isolated airway tissues from neonatal calves (large bronchi, open square [n = 6]; small bronchi, open diamond [6]) and young adult cattle (trachealis muscle, x [8]; large bronchi, black square [6]; small bronchi, black diamond [6]). The amplitude of contractions produced by the agonist is expressed as the percentage of the maximal contraction produced by methacholine (MCh; 10^{-5}M). Data are presented as mean ± SEM. *Value significantly ($P < 0.05$) different from tracheal preparations. †Value significantly ($P < 0.05$) different from results obtained in bronchi of young adult cattle.

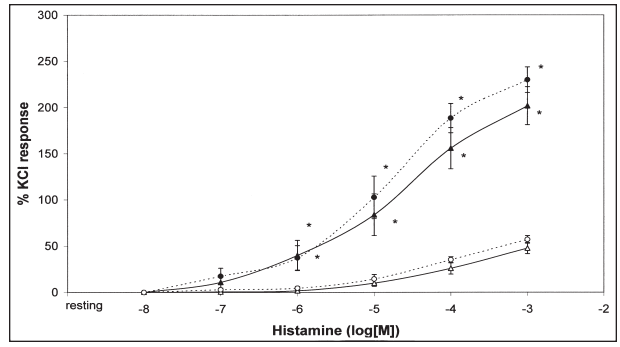

Figure 2—Cumulative dose-response curves to histamine in intrapulmonary artery (open circle or triangle) and vein (closed circle or triangle) samples from 6 neonatal calves (black circle) and 9 young adult cattle (black triangle). The amplitude of contractions produced by the agonist is expressed as the percentage of the maximal contraction produced by KCl (127mM). Data are presented as mean ± SEM. *Value significantly ($P < 0.05$) different from result obtained in intrapulmonary artery samples.

Table 1—Maximal histamine induced-contraction (E_{max}) and potency (pD_2) of large and small bronchi and intrapulmonary arteries (IPAs) and intrapulmonary veins (IPVs) from neonatal calves and young adult cattle

Tissues	Variable	Young adults	Neonatal calves
Large bronchi	E_{max} (× *g*)	0.77 ± 0.42 (6)	2.92 ± 0.86* (6)
	pD_2	NC (6)	4.29 ± 0.16 (6)
Small bronchi	E_{max} (× *g*)	0.35 ± 0.12 (6)	1.57 ± 0.23* (6)
	pD_2	NC (6)	4.57 ± 0.27 (6)
IPA	E_{max} (× *g*)	2.85 ± 0.44 (9)	2.57 ± 0.20 (6)
	pD_2	4.00 ± 0.12 (9)	4.41 ± 0.15 (6)
IPV	E_{max} (× *g*)	9.22 ± 1.26† (9)	7.51 ± 1.43† (6)
	pD_2	4.59 ± 0.11† (9)	4.96 ± 0.15† (6)

Results are expressed as mean ± SEM. Number in parentheses indicates the number of animals sampled.
*Value significantly ($P < 0.05$) different from value obtained in young adult tissue. †Value significantly ($P < 0.05$) different from arterial value in same age group.
NC = Not calculated.

that in IPVs (4.69 ± 0.72 × *g*). However, the maximal contraction of smooth muscle induced by histamine was very much higher in IPVs than in IPAs ($P < 0.001$; Table 1). Maximal contraction of IPVs was 201.8 ± 20.6% of reference KCl-induced contraction, whereas that of IPAs was only 47.6 ± 6.0%. Furthermore, IPV tissue had a higher sensitivity to histamine than did IPA tissue ($P < 0.001$). In neonatal calves, contraction induced by KCl was higher in IPA samples (4.92 ± 0.42 × *g*) than it was in IPV tissue (3.41 ± 0.80 × *g*; $P < 0.05$). However, maximal histamine-induced contractions were 229.9 ± 13.8% and 54.3 ± 3.4% of the reference KCl-induced contraction for IPV and IPA tissues, respectively. These percentages were similar to those obtained from tissues of young adult cattle.

Discussion

In guinea pigs,[14,15] rabbits,[16] dogs,[17] and humans,[18] the peripheral airways are much more sensitive to histamine than the more proximal bronchi or the tracheal muscle. However, in our investigation, a substantial histamine-induced contraction was observed in the trachealis muscle in young adult cattle, but that observed in the bronchi was only slight, even at very high concentrations (10^{-3}M). This lack of response to histamine of bronchi from adult cattle has been reported previously.[19]

The slight contraction of bronchi could be explained by masking of the H1-dependent bronchoconstrictor effect by a powerful H2-dependent bronchodilator effect. A more detailed study of the partitioning of the events induced by histamine between its type 1 and -2 receptors in the bronchi would be required to draw conclusions in this respect. A second explanation would be that inhibitory H3 receptors may be present in the epithelium or smooth muscles of the respiratory tract in cattle, and the density of those receptors may increase gradually with increasing distance from the trachea. It is known that H3 receptors are found in the respiratory tracts of guinea pigs and that their stimulation induces bronchorelaxation.[20,21] Finally, a third explanation may be that, in cattle, the density of the H1 receptors in bronchial smooth muscles declines from the central to the peripheral airways.

In neonatal calves, the amplitude of histamine-induced bronchoconstriction was considerably stronger (48 and 60% of the methacholine-induced constriction for large-B and small-B samples, respectively) than it was in young adults (15 and 20% of the methacholine-induced constriction for large-B and small-B samples, respectively). Preliminary results from similar investigations performed on tissues from 8- to 9-week-old calves have suggested an intermediate response (data not shown). Airway smooth muscle response is known to be affected by increasing age, and characteristically, there is a reduction in response to stimulatory agents.[14,22-24] For instance, age-related reductions in cholinergic responses of bovine trachealis muscle[23] and histaminergic responses of porcine trachea[22] have been described; in the latter tissue, maximal contractile reactivity to histamine declines by approximately 10-fold, as piglets grow into adults. Mechanisms underlying the reduction in contraction and sensitivity in older animals are still unclear. Some data suggest that age-related changes might be associated with a reduced receptor density or properties[25,26] or reduced coupling of receptor to postreceptor processes.[27,28] In young bovine adults, our data suggested that the effects of histamine are restricted to extrapulmonary airways and would result in obstruction and increases in pulmonary resistance. In neonatal calves, histamine induced constriction of both large and small airways, which would induce changes in pulmonary resistance and dynamic compliance.

Potassium causes the smooth vascular muscles to contract by means of membrane depolarisation that is independent of specific receptors.[29] Therefore, the extent of the contraction produced by KCl depends on the active muscular mass.[9] In the study reported here, the extent of the KCl-induced contraction did not differ significantly between IPA and IPV specimens obtained from young adult cattle; thus, the results obtained after the addition of histamine were independent of the methodological aspects of the experiment and may be interpreted in terms of histamine-dependent mechanisms. For the neonatal calves, however, the muscle mass was greater in arteries. The difference in intrinsic contractile capacity of the IPA and IPV samples themselves would have accounted for detection of a stronger arterial contraction in response to histamine. Nevertheless, despite the intrinsically lower muscle mass, veins contracted more than arteries, which confirmed that histamine action would have been even more marked if venous tissue samples had the same intrinsic contractile activity as arteries.

The results of our study indicated that the pulmonary veins react more strongly to histamine than do the arteries, regardless of the age of the animals sampled. This finding is in contradiction of data that indicate that the pulmonary arteries react to histamine much more strongly than do pulmonary veins in rabbits[5] and humans.[6] The results obtained in neonatal calves and young adult cattle in the study reported here are identical in qualitative terms to those obtained in sheep,[7] dogs,[8] and guinea pigs[6,9]; however, they are strikingly different quantitatively. For example, guinea pigs have similar responses to KCl in IPA and IPV samples as those

reported in our study, yet venous contractions in guinea pigs attain approximately 160% of the contraction of arteries, and in the animals of this report, they attained 400% of the contraction of arteries. There may be 2 explanations for the observed difference; arteries may have a lesser density of H1 receptors that initiate contraction or greater relaxant H2 receptor density on their smooth muscle cells, or the endothelium of arteries may produce a greater quantity of relaxing factors that attenuate the contractile response to histamine.[30-33]

In general, our data indicate that histamine affects the respiratory function via the contraction of the trachea and pulmonary veins in young adult cattle, which would increase total pulmonary resistance and induce venous hypertension. It is likely that this phenomenon would also result in increased pulmonary capillary pressure and the development of alveolar edema. It is unlikely, however, that histamine would cause spasms of the more peripheral bronchi via a direct effect on smooth muscle in calves. This conclusion is corroborated by the observation that severe pulmonary oedema is the main pathologic finding after IV administration of histamine to 6-month-old calves.[13]

The results of the study reported here suggest that the main physiopathologic target of pulmonary mast cells is the pulmonary vein in bovids. It is not yet determined if this also applies to other major mediators of inflammation, but it may be speculated that ventilation-perfusion mismatch in the bovine species could originate in the veins, rather than in the arteries. Our data suggest that venodilators may improve the treatment of bovine respiratory diseases, although this remains to be elucidated.

[a]EMKA Technologies, Paris, France.
[b]IT-1 sensor, EMKA Technologies, Paris, France.
[c]Sigma-Aldrich, Bornem, Belgium.
[d]Fluka, Brussels, Belgium.

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