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Regional differences in the mechanical properties of rabbit airway smooth muscle

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1 In studies of rabbit airway smooth muscle, differences in mechanical responses to acetylcholine, histamine and high K^+ in intact muscles, and in Ca^{2+} sensitivity in skinned muscles, have been examined in tissue taken from 5 different regions of the airway. Interactions between prostaglandin $F_{2\alpha}$ and epithio-thromboxane A_2 and the above spasmogenic agencies were also studied.

2 Mechanical responses to histamine (10 μM) and to 128 mM K^+ were smallest in trachea and were largest in 3rd and 4th order bronchi. In all regions, spasm evoked by 10 μM acetylcholine was greater than that evoked by 10 μM histamine or 128 mM K^+.

3 In the third and fourth branches of the rabbit right middle bronchus, contractions evoked by 10 μM acetylcholine, 10 μM histamine and 128 mM K^+ showed similar amplitudes of phasic response. In Ca^{2+}-free solution containing 2 mM EGTA, the phasic components of the acetylcholine- or histamine-induced contraction remained unchanged in comparison with that observed in Krebs solution, but the phasic and tonic components of the K^+-induced contraction and the tonic changes induced by acetylcholine and histamine were abolished.

4 Two subtypes of the histamine receptor, excitatory H_1- and inhibitory H_2- receptors were detected on the bronchial smooth muscle. The H_1-induced contraction was mediated by release of stored Ca^{2+} together with activation of Ca^{2+} influx relatively insensitive to Ca^{2+} antagonists.

5 The $-\log(EC_{50})$ values for acetylcholine and histamine (in the presence of cimetidine and atropine) were 6.11 ± 0.11 and 5.33 ± 0.08, respectively, in the third branch of right middle bronchus. These values were similar to those observed for trachea.

6 Prostaglandin $F_{2\alpha}$ (10 μM) and 9,11-epithio-11,12-methano-thromboxane A_2 (0.1 μM) neither provoked nor enhanced the contractions evoked by any stimulants.

7 No difference was observed between the Ca^{2+} sensitivity of chemically skinned muscle from the trachea and that of muscle from the third branch of the right middle bronchus.

8 Regional differences in the response to histamine and acetylcholine observed in airway smooth muscles are discussed and it is concluded that these may be due to differences in receptor numbers.

Introduction

Although the site of airway obstruction in asthmatics may vary (Despas *et al.*, 1972), many investigators have suggested that during an acute attack, obstruction occurring in large intrapulmonary airways assumes greatest importance for most patients (Epstein *et al.*, 1948; Dulfano *et al.*, 1966; Mildon *et al.*, 1974; Chan-Yeung *et al.*, 1976). There is much evidence to indicate that regional differences exist in the distribution of autonomic nerves between the large and small airways (Hensley *et al.* 1978; Russell, 1978; De Troyer *et al.*, 1979; Barnes *et al.*, 1982; 1983), in their smooth muscle content (Hogg, 1985) and in their electrophysiological properties (Inoue & Ito, 1986). However, because many of the experiments with airway smooth muscles have been carried out on preparations from the trachea, the main bronchus or on parenchymal strips, little is known about the properties of smooth muscles in the lobar, segment or subsegment bronchus (Fleisch & Calkins, 1976; Russell, 1978; Inoue & Ito, 1986).

Of the many substances released from the lung upon antigen challenge, histamine, slow-reacting substance of anaphylaxis (SRS-A) (a mixture of leukotrienes) and prostaglandins seem to be of particular importance in most species. It is known that in a mild asthmatic attack or in the early stages of

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an acute attack in atopic patients, histamine seems to play an important role and anti-histamine agents, especially H_1-antagonists, are effective in these conditions (Nathan *et al.*, 1979).

In the present study, we have attempted to identify differences in the mechanical properties of rabbit bronchial smooth muscles from regions which may well be correlated with the site of bronchoconstriction during an asthmatic attack. For this purpose, we recorded the mechanical responses produced by histamine, acetylcholine and 128 mM K^+ in tissues prepared from the trachea and first, second, third and fourth order bronchi.

Methods

Male albino rabbits (1.8–2.2 kg) were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.v.) and exsanguinated. The trachea and lung were transferred to a dissecting chamber filled with Krebs solution and the trachea and right middle bronchial tree dissected free of parenchyma. Circular smooth muscle strips (0.05–0.08 mm in width and 0.3–0.4 mm in length) were carefully cut out free of cartilage and epithelium.

Solutions

The Krebs solution had the following ionic composition (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.4 and glucose 11.5. High-K^+ solution was prepared by replacing NaCl with KCl isosmotically. Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ with $MgCl_2$ isosmotically and adding 2 mM ethyleneglycol-*bis*-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA). Solutions were bubbled with 97% O_2 and 3% CO_2 and the pH was adjusted to 7.4.

The relaxing solution used in the experiments with skinned preparations had the following composition (mM): K-methanesulphonate (KMs) 130, $Mg(Ms)_2$ 5, Na_2ATP 5, EGTA 4, piperazine-N,N′-*bis*-(2-ethanesulphonic acid) (PIPES) 20, pH 6.8, and the ionic strength was adjusted to 0.17 M with KMs. Solutions containing various concentrations of Ca^{2+} were prepared by adding appropriate amounts of $Ca(Ms)_2$ to the relaxing solution. The precise methods for calculating free ionic concentrations and the binding constants used have been described by Itoh *et al.* (1986).

Recording of mechanical activity

Mechanical responses were measured by attaching a smooth muscle strip prepared from the trachea or bronchus to a strain gauge (U-gauge, Shinko, Tokyo). Both ends of the preparation were fixed between pieces of Scotch double stick tape (3M Co., St Paul, MN). Thin silk thread was used to attach one end of the tissue to the transducer and the other to an anchorage point within the recording chamber (0.9 ml). The strip was stretched to maintain resting tension of about 10 μN. Solutions of modified ionic composition (or containing drugs) were added to the chamber during pumped removal of the solution already present. The test solutions could be changed in a few seconds.

In almost all of the experiments, spasmogenic agents were applied for 3 min at 10–15 min intervals and responses were reproducible with this procedure. Atropine, cimetidine, mepyramine and/or nifedipine were applied 5–10 min before and during application of spasmogens, and washed out rapidly after recording the responses. Such pre-application time was enough to obtain maximal and constant effects. Control responses, recorded before and after each trial, were reproducible (after-controls were not shown in figures). Concentration-response relationships were obtained by application of various concentrations of the spasmogens for 3 min at 10 min intervals.

Skinned tissues were prepared using saponin (25 μg ml^{-1}, for 20 min) according to methods described previously (Itoh *et al.*, 1981; 1986). After a K^+-induced contraction of an intact muscle had been recorded, the bathing solution was replaced with relaxing solution. The preparation was left for 20 min in the relaxing solution containing 25 μg ml^{-1} saponin and washed again with the relaxing solution. To prevent deterioration of the Ca^{2+}-sensitivity of the contractile proteins, 0.1 μM calmodulin was present throughout the experiment. The tension-pCa relationship was obtained by cumulative application of solutions containing various Ca^{2+} concentrations buffered with 4 mM EGTA.

Drugs

The chemicals used in the experiments were saponin (ICN), adenosine 5′-triphosphate (ATP), acetylcholine chloride and indomethacin (Sigma), histamine hydrochloride (Ishizu Pharmac.), 5-hydroxytryptamine (5-HT creatine sulphate, Merck) and atropine sulphate (Merck), cimetidine and mepyramine (Fujisawa), nifedipine (Bayer), prostaglandin $F_{2\alpha}$ and 9,11-epithio-11,12-methano-thromboxane A_2 (Ono), EGTA and PIPES (Dojin). All solutions were freshly prepared before each experiment. The water used in this study was glass-double distilled water and all other chemicals were of the highest reagent grade available.

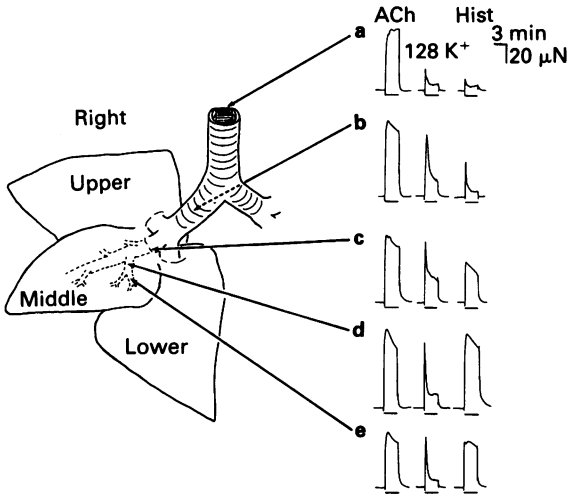

Figure 1 Diagram of the rabbit trachea and bronchial tree showing typical responses to 10 μM acetylcholine (ACh), 128 mM K^+ (128 K^+) and 10 μM histamine (Hist) in different regions. Muscle strips were prepared from the regions indicated. (a) Trachea; (b) right main bronchus; (c) second, (d) third and (e) fourth branch of right middle bronchial tree.

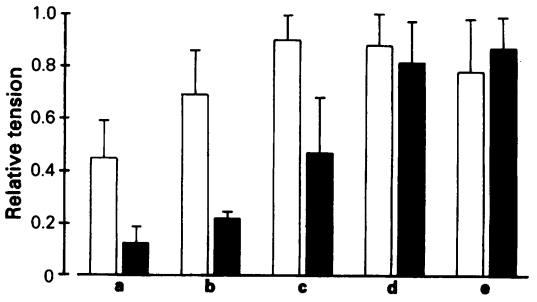

Figure 2 Relative peak amplitudes of the spasm evoked by 128 mM K^+ (open columns) and 10 μM histamine (solid columns) in different regions. The peak amplitude of spasm evoked by 10 μM acetylcholine was normalized as 1.0. (a) Trachea; (b) right main bronchus; (c) second, (d) third and (e) fourth branches of right middle bronchial tree. Vertical lines indicate s.d., $n = 5$–8.

Statistics

The measured values were expressed as the mean $\pm$ s.d. and the number of observations. The significance of differences between means was assessed by use of Student's t test for paired or unpaired values as appropriate.

Results

Regional differences in responses to acetylcholine, histamine and KCl

To investigate the regional differences in responses to various stimulants, the trachea and right middle bronchial tree were used. Figure 1 shows a ventral view of the trachea and right lung of the rabbit and typical mechanical responses to 10 μM acetylcholine (ACh), 128 mM K^+ and 10 μM histamine in each region as indicated by the arrows. From the trachea to the fourth branch of bronchus, absolute values of peak spasm evoked by 10 μM ACh were the same ($40.9 \pm 14.0\,kN\,m^{-2}$ for the trachea and $39.7 \pm 10.5\,kN\,m^{-2}$ for the fourth branch of the middle bronchus; mean $\pm$ s.d., $n = 10$). On the other hand, contractions evoked by high K^+ or 10 μM histamine were smaller in the trachea and main bronchus in comparison to those of peripheral bronchi. 5-HT had no contractile effect in any region.

The peak amplitudes of contraction evoked by 128 mM K^+ or 10 μM histamine were compared with those evoked by 10 μM acetylcholine (normalized as a relative tension of 1.0) in each region (Figure 2). Tracheal smooth muscles exhibited a smaller response to high K^+ in comparison to that evoked by acetylcholine, but in the second, third and fourth branches of bronchus, the peak response was similar to that produced by application of 10 μM ACh (Figure 2c–e). In trachea, responses to 10 μM histamine were smaller than those of the other two stimulants (Figure 2a), but increased towards those of the ACh standard in peripheral airway regions. The maximal response to histamine was recorded in the third and fourth branches of the middle bronchial tree (d–e). Therefore, the third branch of the right middle bronchus was used to characterize the mechanical responses to various stimulants.

The effects of nifedipine and a Ca^{2+}-free medium on responses to acetylcholine, histamine and high K^+

As shown in Figure 3, spasm evoked by 10 μM acetylcholine, 10 μM histamine and 128 mM K^+ comprised phasic and tonic components. To investigate the sources of Ca^{2+} involved in contractions evoked by these stimulants, tissues were exposed to Ca^{2+}-free solution containing 2 mM EGTA. In Ca^{2+}-free conditions, 10 μM acetylcholine and 10 μM histamine provoked only phasic responses. These were not attenuated compared with the phasic responses observed in the presence of Ca^{2+}. High K^+ did not produce a contraction in the Ca^{2+}-free medium. After application of stimulants in Ca^{2+}-free solution,

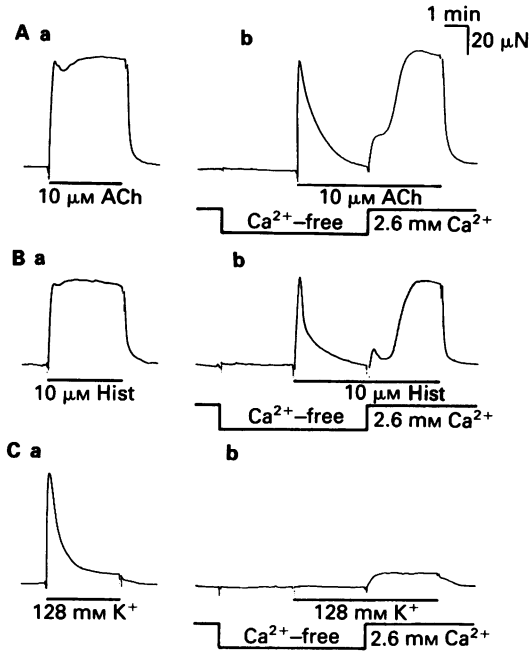

Figure 3 Responses of a bronchial smooth muscle evoked by 10 μM acetylcholine (ACh; A), 10 μM histamine (Hist; B) and 128 mM K^+ (C) in the presence (a) and absence (b) of Ca^{2+} in the superfusate. All responses were recorded from the same preparation obtained from the third branch of a rabbit right middle bronchus. In (b), Ca^{2+}-free solution containing 2 mM EGTA was superfused for 3 min before and during the initial application of the stimulant. Three min after application of each stimulant, 2.6 mM Ca^{2+}-containing solution was again superfused in the presence of the stimulant.

addition of 2.6 mM Ca^{2+} evoked the same amplitude of tonic spasm as that evoked by each stimulant in normal Krebs solution. These results suggested that the K^+-induced spasm was dependent upon extracellular Ca^{2+} and that contractions evoked by acetylcholine or histamine were dependent on Ca^{2+} release from intracellular stores (phasic response) and upon extracellular Ca^{2+} (tonic response).

Although K^+-induced contractions are mainly generated by activation of voltage-dependent Ca^{2+} influx (Bolton, 1979; Kuriyama *et al.*, 1982), some agonists are thought to evoke Ca^{2+} release from storage sites and Ca^{2+} influx via both voltage-dependent and receptor-operated mechanisms (Bolton, 1979). To clarify the possible role of voltage-dependent Ca^{2+} influx, the effects of 0.1 μM nifedipine on contractions evoked by 10 μM acetylcholine, 10 μM histamine and 128 mM K^+ were observed. As shown in Figure 4Ab and Bb, 0.1 μM nifedipine inhibited the tonic response evoked by 10 μM acetylcholine (0.64 $\pm$ 0.03 times the control, $n = 3$) or 10 μM histamine (0.58 $\pm$ 0.04 times the control, $n = 3$) without any attenuation of the phasic response. However, both the phasic and tonic contractions evoked by 128 mM K^+ were markedly but not completely inhibited (phasic: 0.54 $\pm$ 0.09 times the control, $n = 3$ and tonic: 0.11 $\pm$ 0.03, $n = 3$) (4Cb). In the presence of 3 μM atropine, 0.1 μM nifedipine almost completely inhibited the high K^+-induced contraction (4Cd), but it had virtually no effect on contractions evoked by histamine (0.94 $\pm$ 0.09 times, $n = 3$) (4Bd). These results indicate that the direct action of histamine on bronchial smooth muscles results from activation of Ca^{2+} release from intracellular stores and from Ca antagonist-insensitive Ca^{2+} influx. The inhibitory action of nifedipine on the tonic contraction evoked by histamine in the absence of atropine seemed to be due mainly to the inhibition of an indirect action of histamine, which may be mediated by acetylcholine release from nerve terminals.

Effects of atropine, cimetidine and mepyramine on contractions evoked by histamine, acetylcholine and high K^+

To examine whether there was any indirect component in the spasm evoked by the various stimulants, 3 μM atropine was superfused before their application (Figure 5). The acetylcholine (10 μM)-induced spasm was blocked by application of 3 μM atropine. The amplitudes of the phasic and tonic responses evoked by 10 μM histamine were markedly inhibited (phasic: 0.61 $\pm$ 0.11 times the control, $n = 3$, tonic: 0.51 $\pm$ 0.16 times the control, $n = 3$)(B), whilst the phasic response evoked by 128 mM K^+ was slightly inhibited (0.80 $\pm$ 0.03 times the control of phasic response)(C).

Figure 5B also shows the effects of cimetidine and mepyramine on the contractions evoked by 10 μM histamine. Cimetidine (0.1 μM) enhanced contractions evoked by histamine in the presence of 3 μM atropine (1.70 $\pm$ 0.53 times, $n = 3$), and mepyramine (0.1 μM) completely blocked this contraction. Thus, there are H_1- and H_2-receptors on these bronchial smooth muscles. Activation of the H_1-receptor generates muscle contraction, while the H_2-receptor mediates an inhibitory effect.

In tracheal smooth muscles, pretreatment with cimetidine (0.1 μM) also resulted in a small enhancement of the histamine-induced contraction but to a lesser extent than that observed on the bronchial smooth muscles (1.27 $\pm$ 0.06 times, $n = 3$). Thus, the small amplitude of contraction evoked by histamine in the trachea seems not to be due to a dense distribution of the H_2-receptor subtype.

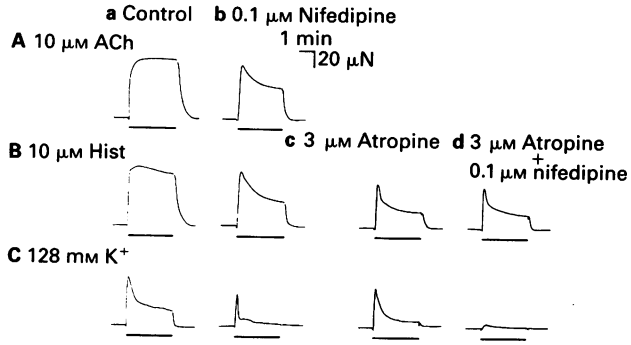

Figure 4 Effects of nifedipine (0.1 μM) on spasm evoked by 10 μM acetylcholine (Ach; A), 10 μM histamine (Hist; B) and 128 mM K^+ (C). All responses were recorded from the same preparation obtained from the third branch of the rabbit right middle bronchus. (a) Control, (c) 3 μM atropine, (b) and (d) with application of nifedipine in the presence (d) and absence (b) of 3 μM atropine. Nifedipine was applied for more than 10 min before and during application of stimulants. Between (b) and (c), nifedipine was effectively removed and responses similar to the control were observed before tissue exposure to atropine.

Figure 6 shows the concentration-response curves for histamine (a) and acetylcholine (b) on the trachea and the third branch of middle bronchus. In the case of the histamine-induced contraction, 3 μM atropine with 0.1 μM cimetidine was added. The $-\log(EC_{50})$ values were 6.11 ± 0.10 ($n = 5$) for acetylcholine and 5.33 ± 0.08 ($n = 5$) for histamine in the third branch of bronchus. These values were not different from those obtained in the trachea (6.11 ± 0.11, $n = 5$ for acetylcholine and 5.27 ± 0.08, $n = 5$ for histamine).

Effects of prostaglandin $F_{2\alpha}$, thromboxane A_2 and indomethacin

In rabbit airway smooth muscle (the third branch of the middle bronchus), prostaglandin $F_{2\alpha}$ (10 μM) and 9,11-epithio-11,12-methano-thromboxane A_2 (0.1 μM) neither provoked contraction themselves nor enhanced those evoked by any concentration of acetylcholine (0.1–100 μM), histamine (0.1–100 μM) or high K^+ (48 mM–128 mM) (Figure 7a and b). Further, 1 μM indomethacin did not modify contractions evoked by any concentration of acetylcholine, histamine or high K^+ (c). No effect of these prostanoids and indomethacin on the contractions evoked by these three stimulants was observed in the trachea.

Ca^{2+} sensitivity of chemically skinned smooth muscles

In an attempt to clarify the nature of the observed regional differences, the Ca^{2+} sensitivity of the contractile proteins was investigated using saponin-

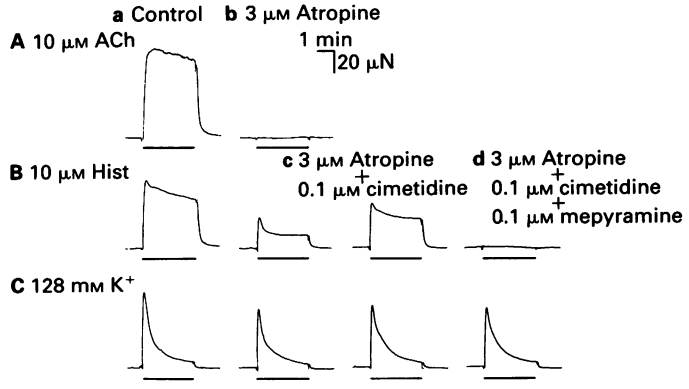

Figure 5 Effects of atropine (3 μM), atropine and cimetidine (0.1 μM), and atropine, cimetidine and mepyramine (0.1 μM) on the spasm evoked by 10 μM acetylcholine (ACh; A), 10 μM histamine (Hist; B) or 128 mM K^+ (C). All responses were recorded from the same preparation obtained from the third branch of the rabbit right middle bronchus. Atropine, cimetidine or mepyramine was applied for 5 min before and during application of stimulants.

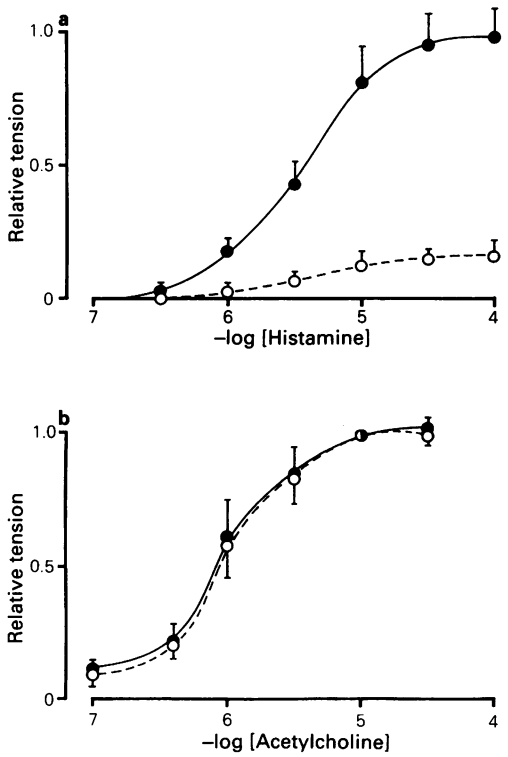

Figure 6 Log concentration-response relationships for histamine (a) and acetylcholine (b) on the trachea (○) and the third branch of the rabbit right middle bronchus (●). The contraction evoked by 10 μM acetylcholine was normalized as 1.0 in both (a) and (b). The histamine-induced contraction was measured 10 min after application of 3 μm atropine and 0.1 μm cimetidine. Vertical lines indicate s.d., $n = 5$.

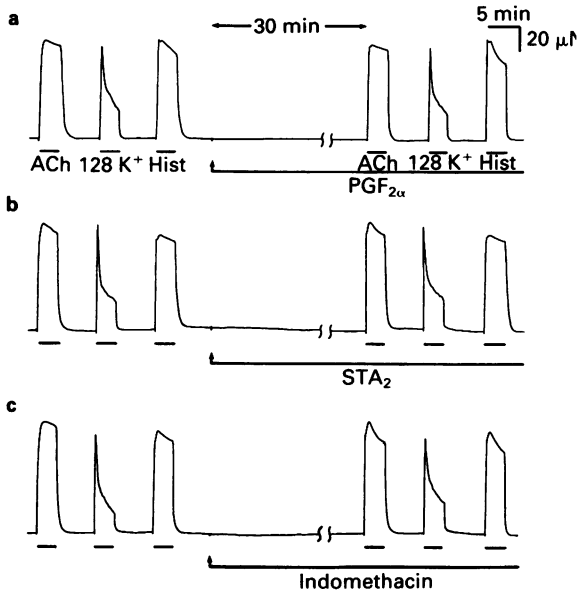

Figure 7 Effects of 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; a), 0.1 μM 9,11-epithio-11,12-methano-thromboxane A_2 (STA_2; b) and 10 μM indomethacin (c) on the third branch of the rabbit right middle bronchus. Spasms evoked by acetylcholine (10 μM), histamine (10 μM) and 128 mM K^+ were observed before and 30 min after application of each of the modifying agents. (a), (b) and (c) were recorded from different preparations.

treated skinned muscle tissues. As shown in Figure 8, there were no differences in the Ca^{2+} sensitivity of muscle from the trachea and muscle from the third branch of middle bronchus, i.e. the minimum concentration of Ca^{2+} required to produce a contraction was 0.1 μM and a maximum contraction was obtained by application of 10 μM Ca^{2+} in both tissues. $-\text{Log}(EC_{50})$ values for the Ca^{2+}-induced contraction were 6.07 ± 0.07 ($n = 5$) in the trachea and 6.16 ± 0.06 ($n = 5$) in the third branch, respectively. Therefore, the observed regional differences in the mechanical responses were not apparently due to fundamental differences in the contractile process.

Discussion

In the present study, responses to acetylcholine, histamine and high K^+ varied from region to region, but acetylcholine consistently produced the largest mechanical changes in every region studied. In all areas, the pattern of spasmogenic response was broadly similar and consisted of an initial phasic component followed by a tonic one. To analyse these effects, a detailed study was made of spasm generated in the third branch of the middle bronchus. It will be assumed that conclusions drawn from this localized investigation are generally applicable to responses obtained in other regions.

Analysis of the mechanisms by which contractions to acetylcholine, histamine or high K^+ were produced was often complicated by indirect components in these responses. In the case of acetylcholine, spasm was abolished by atropine and it is concluded that this was mediated by muscarinic receptors located on smooth muscle cells. Tension development induced by high K^+ was partly inhibited by atropine but not modified by either mepyramine or cimetidine. Thus significant depolarization of cholinergic nerve terminals was induced by high K^+. The situation with histamine was more complicated. Cimetidine, the H_2-receptor blocker, enhanced and mepyramine, the H_1-receptor blocker, inhibited histamine-induced contractions. Presumably the two histamine receptor subtypes (H_1: Ash & Schild, 1966; H_2: Black *et al.*, 1972) contribute to the final contraction *in vivo*, but it seems that the H_1-receptor may be more predominantly distributed than the

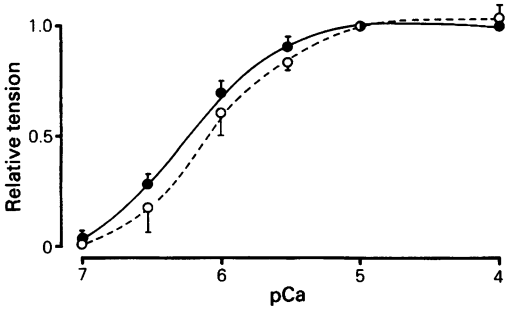

Figure 8 The Ca^{2+} sensitivity of chemically skinned airway smooth muscles excised from the trachea (○) and the third branch of bronchus (●) using 25 $\mu g\,ml^{-1}$ saponin for 20 min. Various concentrations of Ca^{2+} (pCa; from low to high) were cumulatively applied. The peak amplitude of the 10 μM Ca^{2+}-induced spasm was normalized as a relative tension of 1.0 and was 1.23 ± 0.11 times the peak amplitude of 10 μM ACh-induced spasm in trachea and 1.45 ± 0.20 times in third branch of bronchus, respectively. Each point represents the mean and vertical lines indicate s.d., $n = 3$–5.

H_2-subtype. A heterogeneous distribution of both subtypes was observed in the present study from the trachea to the fourth branch of the bronchus. A small contraction evoked by histamine in the trachea was not due to a dense distribution of the H_2-subtype, because after application of cimetidine the amplitude of histamine-induced contractions was only slightly enhanced. H_1-receptor-induced contractions and H_2-receptor-induced relaxations in smooth muscle tissues are not features specific to airway smooth muscles. They are observed in many preparations including those of vascular tissue (Hirschowitz, 1979).

It is of interest that atropine inhibited the histamine-induced contraction. Atropine may inhibit the effect of acetylcholine released from cholinergic nerve terminals, as estimated from the amplitude of excitatory junction potentials recorded from tracheal smooth muscle by Inoue & Ito (1986), and partially inhibit the histamine receptor (Weiner, 1980).

Using Ca^{2+}-free conditions and the Ca^{2+}-entry blocking drug, nifedipine, the mechanisms associated with the directly mediated contractions produced by the three stimulants were investigated. Both the phasic and the relatively small tonic components of the high K^+-induced contraction were essentially blocked by Ca^{2+} removal or by nifedipine, indicating that both were associated with Ca^{2+} influx through voltage-dependent channels. However, responses to acetylcholine and histamine were complex. The phasic contractions evoked by acetylcholine and histamine were not modified in Ca^{2+}-free solution but the tonic responses to the agonists were abolished. In the presence of nifedipine, these tonic contractions were reduced but not abolished. Thus, the phasic component of acetylcholine- or histamine-induced contractions is mainly due to the release of Ca^{2+} from cellular storage sites, while the tonic component is maintained by both nifedipine-sensitive and insensitive Ca^{2+} influx.

In some smooth muscles, there are two populations of Ca^{2+} current, one sensitive and the other insensitive to nifedipine. The former seem to be important when the cell is maximally activated, as measured using patch and single cell voltage clamp methods (Loirand *et al.*, 1986; Sturek & Hermsmeyer, 1986). We did not measure the electrical responses induced by acetylcholine and histamine. However, since nifedipine partly inhibited the tonic response, it seems that the tonic component of the acetylcholine- and histamine-induced spasm may be mainly due to activation of receptor-activated Ca^{2+} influx (Bolton, 1979; Van Breemen *et al.*, 1979; Kuriyama *et al.*, 1982) with some contribution from nifedipine-sensitive Ca^{2+} influx.

When concentration-response relationships were calculated, the EC_{50} for the spasm induced by acetylcholine or histamine was the same in both the trachea and third order bronchus. Furthermore, when the pCa-tension relationship was observed in skinned muscle tissues excised from these regions, the concentrations of Ca^{2+} required to produce minimum and maximum mechanical responses, respectively, were the same in both tissues. Thus, the basic contractile properties in all regions of the airway smooth muscle may be the same. The different responsiveness to histamine seen may be due to differences in the density of distribution of the H_1-subtype but not of the H_2-subtype, since contractions evoked by histamine in the trachea and the third branch of bronchus were only slightly enhanced and to a similar extent after pretreatment with cimetidine. In human airway, it was reported that the number of smooth muscle cells per unit area increases from the trachea to the terminal bronchus (Hogg, 1985). This may also contribute to regional differences, though in rabbit tissue, regional differences in responses to acetylcholine were not observed.

In vivo, the elastic and resistance tissue components other than smooth muscle may also modify mechanical responses and contribute to regional differences, e.g. the walls of rabbit airway contain substantial cartilage in the trachea and down to second order bronchi, but the third order bronchus contains only small fragments of cartilage. Thus contractions evoked in peripheral regions by histamine and acetylcholine may be more important in the regulation of airway resistance. In earlier studies in tracheal smooth muscles, the contraction and excitatory junction potentials evoked by peripheral cholinergic nerve stimulation gradually declined but could be

restored by application of indomethacin. Prostanoids inhibited the excitatory junction potential and subsequently generated contraction in the presence and absence of indomethacin with slight modification of the agonist-induced contraction (Ito & Tajima, 1981a,b; Inoue & Ito, 1985). In the present study, the tissues excised from the bronchus were not modified by prostaglandin $F_{2\alpha}$, thromboxane derivatives or indomethacin. Thus, the distribution of prostanoid receptors may not vary in the trachea and bronchus; they may be located mainly on nerve terminals and regulate transmitter release.

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