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Nonadrenergic, noncholinergic responses stabilize smooth muscle tone, with and without parasympathetic activation, in guinea-pig isolated airways

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Nonadrenergic, noncholinergic responses stabilize smooth muscle tone, with and without parasympathetic activation, in guinea-pig isolated airways. A. Lindén, C-G. Löfdahl, A. Ullman, B-E. Skoogh. ©ERS Journals Ltd 1993.

ABSTRACT: In guinea-pig isolated airways, nonadrenergic, noncholinergic (NANC) neural responses converge towards a similar level of smooth muscle tone, *via* a contraction when the tone is low prior to stimulation, and *via* a relaxation when the tone is high prior to stimulation. We wanted to assess the effect of simultaneous parasympathetic activation on these converging NANC responses, with and without the addition of sympathetic activation.

In guinea-pig isolated airways, the spontaneous airway tone was initially abolished by indomethacin (10 μM). In one series, adrenergic depletion by guanethidine (10 μM) was then established, with and without cholinergic blockade by atropine (1 μM). In another series, either cholinergic blockade by atropine (1 μM) or no blockade was utilized. Responses to electrical field stimulation (1,200 mA, 0.5 ms, 3 Hz for 240 s) were studied with no induced tone, at a moderate (0.3 μM) and at a near-maximum (6 μM), histamine-induced tone.

The mean level of the tonus equilibrium (% of maximum tone) was higher with the simultaneous NANC and parasympathetic activation than with NANC activation alone (75% compared with 44%, in the main bronchus, n=8). The level of the tonus equilibrium was also higher with the simultaneous NANC, sympathetic and parasympathetic activation than with NANC and sympathetic activation only (49% compared with 21%, in the main bronchus, n=8). The pattern was similar in the distal trachea.

In conclusion, NANC neural responses can stabilize smooth muscle tone, and this stabilizing effect can be modulated by both parasympathetic and sympathetic activation, in guinea-pig isolated airways.

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In human airways, the relaxant neural influence on smooth muscle tone appears to be mediated primarily by nonadrenergic nerves [1–5]. Damage to these nerves might, therefore, contribute to severe smooth muscle contraction, as indicated by a recent report on the absence of the putative nonadrenergic relaxant transmitter, vasoactive intestinal polypeptide (VIP), in asthmatic subjects [6].

An increase in the contractile, noncholinergic neural influence on tone [7] could also contribute to severe smooth muscle contraction, as suggested by a recent report on the increase in the putative noncholinergic contractile transmitter, substance P (SP), in asthmatic subjects [8]. It is well known that a corresponding effect is produced by an increased parasympathetic influence on tone [9–12]. The maintenance of a balance between the contractile and the relaxant neural influences on smooth muscle tone could, therefore, be of potential pathogenetic importance in obstructive airways disease, and deserves characterization.

In guinea-pig isolated airways, it appears that the balance between the contractile and the relaxant nonadrenergic, noncholinergic (NANC) neural responses has a stabilizing effect on tone [13, 14]. When the tone prior to activation is low, NANC activation evokes a contraction, and when the tone prior to activation is high, NANC activation evokes a relaxation. The contractile and relaxant NANC responses thus converge towards a similar level of tone - a NANC convergence effect. It is not known, however, how this convergence effect relates to parasympathetic activation, which might be of interest since the parasympathetic influence on tone is thought to be significant [15].

The aim of the present study was to examine the effect of parasympathetic activation on the ability of NANC responses to stabilize tone. We also examined whether

this parasympathetic effect can be abolished by simultaneous sympathetic activation.

Methods

Airway preparation

Twenty female Dunkin-Hartley guinea-pigs (300–500 g) were killed by cervical dislocation and exsanguination. The thoracic contents were removed and placed in a 200 ml oxygenated (94% O_2; 6% CO_2) dissection bath, filled with buffered nutrient solution (Krebs-Ringer), with the following composition (mM): NaCl (118); KCl (5.9); $CaCl_2$ (2.5); $MgSO_4$ (1.2); NaH_2PO_4 (1.2); $NaHCO_3$ (25.5) and glucose (5.6), at room temperature. The distal (intrathoracic) trachea and the main bronchi were dissected free, and two airway rings were then cut transversely from each airway segment, each containing four to five cartilaginous rings. The airway rings were opened longitudinally along the anterior, cartilaginous part and connected to steel hooks as isolated strips. These preparations were mounted vertically in temperature-controlled (37°C) and oxygenated organ baths (8 ml), which were continuously flooded with fresh Krebs-Ringer solution (0.4 ml·min^{-1}). This handling leaves the airways histologically intact [16].

Tension recordings

The semi-isometric tension was continuously recorded by connecting the preparations to Grass force transducers (FT03). The signals from these transducers were digitally transformed in an NB-MIO-16 converting board and registered in a Macintosh II computer, using the LabVIEW signal-processing software (National Instruments, Austin, Texas, USA), [17].

Electrical field stimulation

Constant current generators provided electrical field stimulation (EFS) *via* rectangular platinum electrodes (5×30 mm) mounted in parallel (10 mm apart), with the airway preparations in between. Square biphasic electrical impulses of 0.5 ms pulse duration were delivered at a current of 1,200 mA [16]. For control contractions alone, a stimulation frequency of 12 Hz was used and a total duration of 20 s.

Selective NANC activation was performed using a frequency of 3 Hz and a total duration of 240 s, with atropine (1 μM) and guanethidine (10 μM) present, in order to obtain cholinergic blockade [18], and adrenergic depletion [2, 19]. These parameters produce submaximum NANC responses sensitive to tetrodotoxin, thereby confirming a neural origin [16].

The simultaneous activation of NANC and parasympathetic responses was performed, using the same stimulation parameters as for selective NANC activation, but in the presence of guanethidine alone. The same stimulation parameters were also used for the simultaneous activation of NANC, sympathetic and parasympathetic responses but, in this case, without atropine and guanethidine. When atropine and guanethidine were added, they were present continuously throughout the experiments.

Experimental protocols

General procedures. In all experiments, the spontaneous airway tone was initially abolished by indomethacin (10 μM) [20], which was continuously present in the Krebs-Ringer solution. A passive tension of 1.2 g was applied to the preparation during 25 min of initial equilibration. A control contraction was then evoked by EFS in order to check the viability of the airways preparation. The Krebs-Ringer solution was then exchanged by emptying the organ bath and, thereafter, refilling it with fresh Krebs-Ringer solution (wash-out). The passive tension was readjusted to 1.2 g, which is the optimum level for contractile responses to EFS both in the trachea and in the main bronchus (data not shown). After this adjustment of passive tension, the protocols differed (see below). All of the experiments ended, however, with continuous flow of Krebs-Ringer solution over 25 min, followed by a maximum histamine-induced (0.1 mM) contraction without any Krebs-Ringer flow.

Frequency response characteristics for the contractile NANC response with and without simultaneous parasympathetic activation. These experiments were performed in order to examine whether the parasympathetic response and the noncholinergic contractile response have different frequency response characteristics. In these experiments alone, the responses were related to, and expressed as, the percentage of the maximum contractile response to EFS in each airway preparation. Using this transformation, the additional parasympathetic contraction was compensated for.

The main bronchus was used [13, 16]. Two bronchial preparations were taken from each guinea-pig. One of these preparations was treated with guanethidine and atropine whereas the other was treated with guanethidine alone. This drug treatment was performed after the initial control contraction and adjustment of applied tension. Seventy minutes of drug incubation followed and then, without any induced tone prior to stimulation, a series of electrical field stimulations (240 s) was performed in the following order: 0.3, 1, 3, 10, 30 Hz (n=2) or 30, 10, 3, 1, 0.3 Hz (n=2). A wash-out followed each field stimulation, and the tone was allowed to return to the prestimulatory level over 60 min before the subsequent stimulation.

Stabilizing effect of NANC responses with and without simultaneous parasympathetic activation. In these experiments, we examined the effect of simultaneous parasympathetic activation on the ability of NANC responses to stabilize tone. The distal trachea and the main

bronchus were used [13, 16]. Two tracheal and two bronchial preparations were taken from each guinea-pig. One of the tracheal and one of the bronchial preparations was treated with guanethidine and atropine whereas the other corresponding preparation was treated with guanethidine alone. This drug treatment was performed after the initial control contraction and adjustment of applied tension. Seventy minutes of drug incubation followed and EFS was then performed (3 Hz for 240 s) without any induced tone prior to stimulation. This stimulation was followed by a wash-out and 25 min of flushing. The tone prior to stimulation was then increased to a moderate level by histamine (0.3 μM). The tone stabilized during a period of 20 min and then remained stable. Another EFS (3 Hz for 240 s) was performed, followed by a wash-out and 25 min of flushing. The tone prior to stimulation was then increased to a near-maximum level by histamine (6 μM). The tone stabilized during a period of 20 min and a final EFS (3 Hz for 240 s) followed.

These experiments, and the subsequent ones, included three repeated electrical field stimulations. In separate experiments, we therefore ascertained that the added parasympathetic and NANC responses, as well the NANC responses alone, do not display any tachyphylaxis, which could have explained the results of the present study (data not shown).

Stabilizing effect of NANC and sympathetic responses with and without simultaneous parasympathetic activation. In these experiments, we examined the effect of simultaneous parasympathetic activation on the added ability of NANC and sympathetic responses to stabilize tone.

The protocol of these experiments was identical to the previous series on the convergence effect (above) with one exception: after the initial control contraction, atropine or no drug was added.

Drugs

Atropine sulphate (Sigma), guanethidine sulphate (Ciba-Geigy) and histamine dichloride (Merck) were dissolved in Krebs-Ringer solution. Indomethacin (Dumex, Confortid, 5 $mg \cdot ml^{-1}$) was diluted in Krebs-Ringer solution. All concentrations (mM) refer to the final organ bath concentration after dilution in the Krebs-Ringer solution.

Data analysis

Tone levels. The tension was measured in grams (1 g = 9.81 mN). The total (active plus passive) tension [20] was measured immediately before and at the end of each EFS and, in some experiments, 25 min after each stimulation. The tension level in the resting preparation, prior to the EFS (1,200 mA, 3 Hz) performed without induced tone, was regarded as purely passive tension [20]. Within each airway preparation, the difference in tension between the passive level and the peak level during the maximum histamine-induced contraction (0.1 mM) was regarded as the maximum active tension. Within each preparation, all of the levels of active tension (tone) were then related to, and expressed as, a percentage of this maximum active tension (% of maximum tone).

Convergence effect. This term was used to describe the added magnitude of the converging contractile and relaxatory responses to EFS [13]. The convergence effect was defined on the basis of the difference between the lowest level of tone (no histamine) and the highest level of tone (histamine 6 μM) established prior to EFS. The convergence effect was then calculated as the decrease in this difference in tone induced by the converging contractile and relaxant responses.

Tonus equilibrium. This estimation of the level of tone towards which the responses to EFS converged [13] reflects the balance between the contractile and relaxatory responses. The tonus equilibrium was calculated within each preparation as the mean of the tone levels at the end of the three electrical field stimulations evoked at different levels of tone.

Statistical evaluation. Data were expressed as arithmetic mean (SEM). Student's t-distribution for differences between data was determined using two-tailed distribution for all comparisons, except the data on the parasympathetic effect on the tonus equilibrium. In this case, the one-tailed distribution was used, since the outcome could be either no effect or an increase in the tonus equilibrium. This was done because there is no relaxant cholinergic parasympathetic effect, as indicated by our frequency response experiments. We used 95% confidence intervals for comparisons including data compared once. However, for comparisons of data on the convergence effect and the tonus equilibrium, we used 99% confidence intervals. This was done because the maximum active tension within each airway preparation was included in three comparisons. It should be noted that this technique is more conservative than the Bonferroni method [21]. Unless otherwise stated, the comparisons were based on paired data (see Experimental protocols).

Results

Characteristics of responses to electrical field stimulation

Without tone prior to EFS, selective NANC activation induced a contractile response with one dominant phase in the main bronchus (fig. 1A). An increase in the tone prior to stimulation, caused by the addition of histamine (0.3–6 μM), reversed the NANC contraction into a relaxation in all 8 preparations. The simultaneous activation of the parasympathetic response did not change this pattern, but it increased the contraction and reduced the relaxation (fig. 1B).

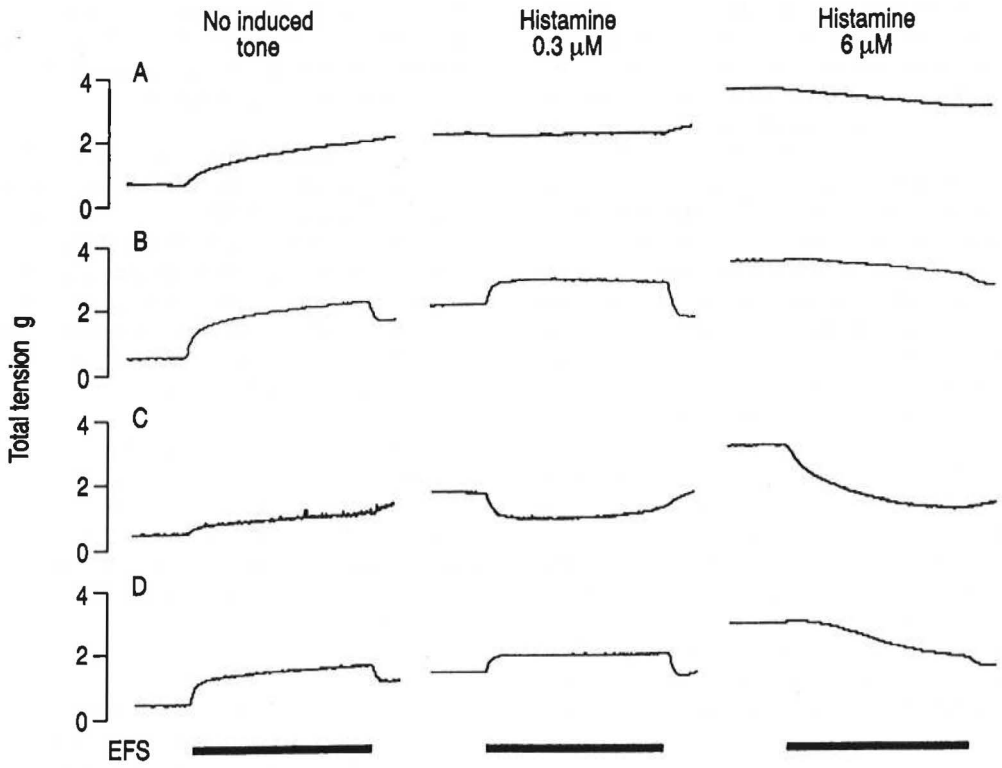

Fig. 1. – Original tracings from the guinea-pig isolated main bronchus showing the response to electrical field stimulation (EFS) (indicated by horizontal bars: 1200 mA, 0.5 ms, 3 Hz for 240 s) at a gradually increased histamine-induced (0, 0.3 and 6 μM) tone in the presence of guanethidine (10 μM), with (A) or without (B) atropine (1 μM). The corresponding responses in preparations without guanethidine, with (C) and (D) without atropine (1 μM), are also shown. All preparations were pretreated with indomethacin (10 μM).

The reversal of the contractile response into a relaxation, caused by the increased tone prior to activation, was also observed with the simultaneous activation of the sympathetic and the NANC response. However, this combination increased the relaxation and reduced the contraction (fig. 1C) but, again, the addition of simultaneous parasympathetic activation increased the contraction and reduced the relaxation (fig. 1D).

The distal trachea demonstrated a pattern similar to the main bronchus (tracings not shown).

Frequency response characteristics for the contractile NANC response, with and without simultaneous parasympathetic activation

At 3 Hz, the responses were submaximum in both groups (fig. 2). At this frequency, the added parasympathetic and NANC contractile response demonstrated a higher degree of activation in comparison with selective NANC activation. The mean(SEM) difference was 17(3)% of maximum response to EFS and proved to be statistically significant (95% confidence interval for difference: 8 to 26% of maximum response to EFS, n=4). There was a similar trend at lower stimulation frequencies.

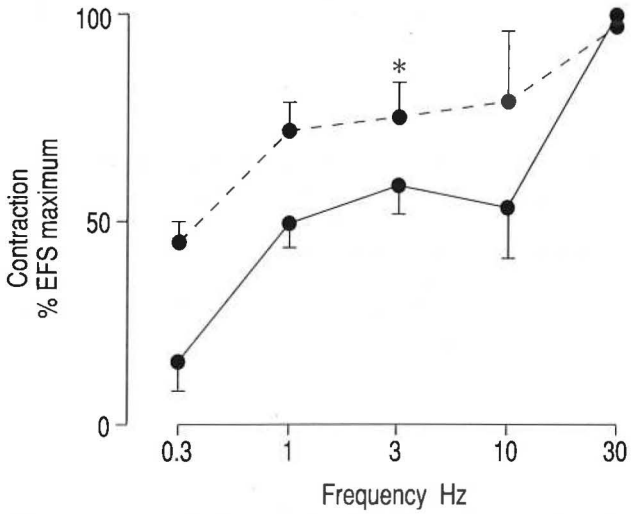

Fig. 2. – Frequency response characteristics from the guinea-pig isolated main bronchus using electrical field stimulation (EFS) (1,200 mA, 0.5 ms, 0.3–30 Hz for 240 s) without any induced tone prior to stimulation. Guanethidine (10 μM) was continously present with (solid lines) or without (dashed lines) atropine (1 μM). All preparations were pretreated with indomethacin (10 μM). In these experiments, the responses are presented as the mean (with SEM-bar) percentage of the maximum contraction induced by EFS for each treatment (% EFS maximum). *: $p<0.05$.

Stabilizing effect of NANC responses, with and without simultaneous parasympathetic activation

Convergence effect. Without tone prior to EFS (no histamine), a contraction was evoked. At the highest level of induced tone (histamine 6 μM) prior to stimulation, a relaxation was evoked. These converging contractile and relaxant NANC responses markedly reduced the difference in tone established prior to stimulation - a "convergence effect" (fig. 3). The mean(SEM) magnitude of this NANC convergence effect (table 1) was increased by 2(6)% of maximum tone by simultaneous parasympathetic activation in the distal trachea. In contrast, the magnitude of the NANC convergence effect (table 1) was reduced by 9(9)% of maximum tone by simultaneous parasympathetic activation in the main bronchus. These differences were not, however, statistically significant in either the distal trachea (99% confidence interval for difference: -20 to 23% of maximum tone, n=8) or the main bronchus (99% confidence interval for difference: -39 to 20% of maximum tone, n=8). After EFS, the tone returned to a level similar to that prior to stimulation.

Tonus equilibrium. The mean(SEM) level of tone towards which the NANC responses converged - the tonus equilibrium (table 2) - was increased by simultaneous parasympathetic activation by 36(5)% of maximum tone in the distal trachea, and by 31(6)% of maximum tone in the main bronchus. This increase was statistically significant both in the distal trachea (99% confidence interval for difference: 22 to 49% of maximum tone, n=8) and in the main bronchus (99% confidence interval for difference: 13 to 49% of maximum tone, n=8).

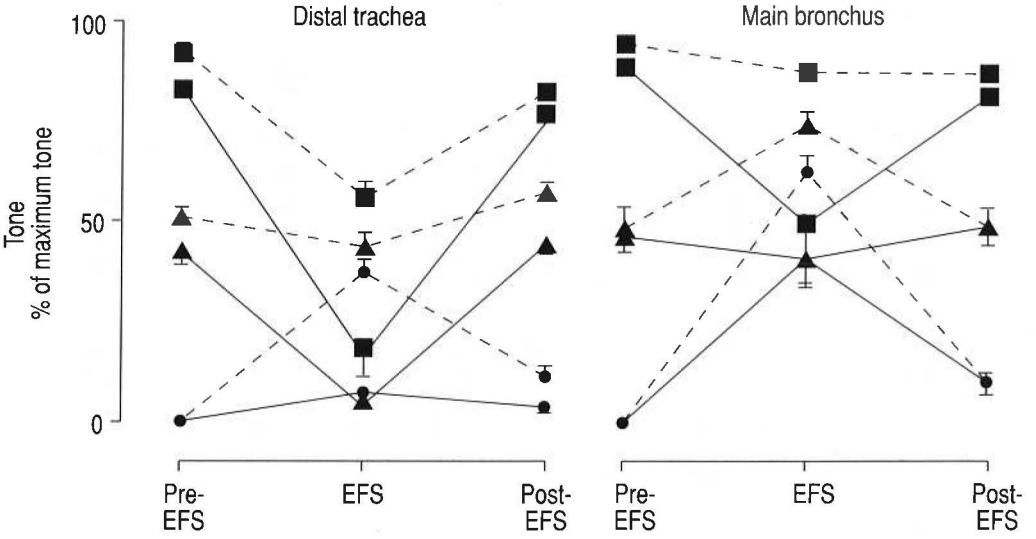

Fig. 3. – Response to electrical field stimulation (EFS) (1,200 mA, 0.5 ms, 3 Hz for 240 s) in guinea-pig isolated airways, at various levels of tone prior to stimulation: no tone in the presence of indomethacin (10 μM) (●), a moderate tone caused by addition of histamine (0.3 μM) (▲), and a high tone caused by more histamine (6 μM) (■). Guanethidine (10 μM) and atropine (1 μM) (solid lines), or guanethidine (10 μM) alone (dashed lines), were continuously present. The data are presented as the mean (with SEM-bar) percentage of the maximum histamine-induced (0.1 mM) tone (% of maximum tone) (n=8).

Table 1. – Convergence effect (% of maximum tone) induced by contractile and relaxant responses to electrical field stimulation in guinea-pig isolated airways

	n	NANC		NANC+ parasympaticus	NANC+ sympaticus		NANC+ sympaticus+ parasympaticus
		%		%	%		%
Distal trachea	8	73 (7)	- NS -	74 (5)	69 (6)	- NS -	79 (3)
Main bronchus	8	79 (6)	- NS -	70 (4)	73 (4)	- NS -	78 (7)

Convergence effect: there was a difference of 70–80% of maximum tone between the lowest and highest level of tone established prior to electrical field stimulation (1,200 mA, 0.5 ms, 3 Hz for 240 s). The converging contractile and relaxant responses to electrical field stimulation reduced this difference in tone - a convergence effect (mean(SEM)). Maximum tone: induced by 0.1 mM of histamine. NANC: treatment by guanethidine (10 μM) and atropine (1 μM); NANC+parasympaticus: treatment by guanethidine (10 μM); NANC+sympaticus: treatment by atropine (1 μM); NANC+sympaticus+parasympaticus: no treatment by atropine or guanthidine; NS $p>0.05$ (Student's t-distribution, two-tailed, paired data), n: number of independent comparisons (see methods); NANC: nonadrenergic, noncholinergic.

Table 2. – The level of tone (% of maximum tone) towards which responses to electrical field stimulation converge - the tonus equilibrium - in guinea-pig isolated airways

	n	NANC		NANC+ parasympaticus	NANC+ sympaticus		NANC+ sympaticus+ parasympaticus
		%		%	%		%
Distal trachea	8	10 (2)	- * -	46 (3)	2 (1)	- NS -	16 (5)
Main bronchus	8	44 (8)	- * -	75 (2)	21 (3)	- * -	49 (2)

Tonus equilibrium (mean (sem)): the mean level of tone at the end of the three electrical field stimulations (1,200 mA, 0.5 ms, 3 Hz for 240 s) evoked at various levels of tone. *: $p<0.05$; NS=$p>0.05$ (Student's t-distribution, one-tailed, paired data). For abbreviations and details see legend to table 1.

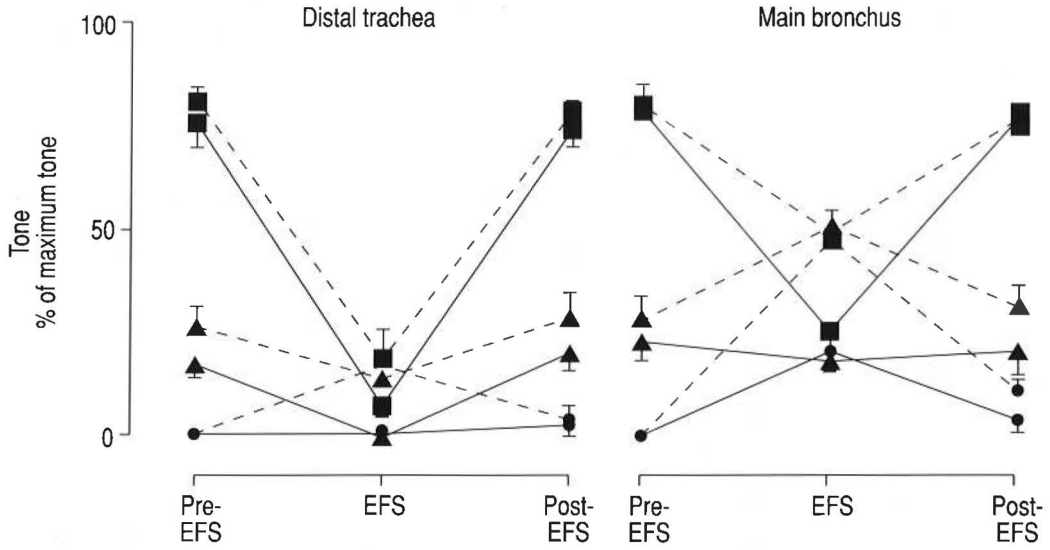

Fig. 4. – Responses to electrical field stimulation (EFS) (1,200 mA, 0.5 ms, 3 Hz for 240 s) in guinea-pig isolated airways, at various levels of tone prior to stimulation. These data were obtained as described in figure 3. The responses to EFS were evoked in the presence of atropine (1 μM) (plain lines) or without atropine (dashed lines). The data are presented as in figure 3 (n=8).

In the different groups of these experiments, the mean(SEM) maximum histamine-induced (0.1 mM) active tension ranged from 2.14(0.17) to 3.09(0.37) g, without any systematic difference which could be attributed to the drug treatment.

Stabilizing effect of NANC and sympathetic responses, with and without simultaneous parasympathetic activation

Convergence effect. A convergence effect was also produced by the added NANC and sympathetic responses (fig. 4). The magnitude of this convergence effect (table 1) was increased by simultaneous parasympathetic activation by 10(6)% of maximum tone in the distal trachea and by 5(7)% of maximum tone in the main bronchus. This increase was not, however, statistically significant either in the distal trachea (99% confidence interval for difference: -12 to 32% of maximum tone, n=8) or in the main bronchus (99% confidence interval for difference: -20 to 30% of maximum tone, n=8). After EFS, the tone returned to a level similar to that prior to stimulation (fig. 4).

Tonus equilibrium. The level of tone towards which the added NANC and sympathetic responses converged - the "tonus equilibrium" (table 2) - was increased by simultaneous parasympathetic activation by 14(5)% of maximum tone in the distal trachea and by 28(4)% of maximum tone in the main bronchus. This increase was statistically significant in the main bronchus (99% confidence interval for difference: 16 to 39% of maximum tone, n=8), but not in the distal trachea (99% confidence interval for difference: -2 to 30% of maximum tone, n=8).

In the different groups of the current experiments, the mean(SEM) maximum histamine-induced (0.1 mM) active tension levels ranged from 2.61(0.28) to 3.07(0.28) g, without any systematic difference which could be attributed to the drug treatment.

Effect of sympathetic activation on the tonus equilibrium (unpaired comparisons)

In contrast to parasympathetic activation, simultaneous sympathetic activation reduced the level of the tonus

equilibrium towards which the NANC responses converged. The magnitude of this reduction was 8(3)% of maximum tone in the distal trachea, and 23(8)% of maximum tone in the main bronchus. The reduction was statistically significant both in the distal trachea (99% confidence interval for difference: 1 to 15% of maximum tone, n=8) and in the main bronchus (99% confidence interval for difference: 2 to 44% of maximum tone, n=8).

The level of the tonus equilibrium towards which the added parasympathetic and NANC responses converged was also reduced by simultaneous sympathetic activation. The magnitude of this reduction was 30(6)% of maximum tone in the distal trachea and 27(3)% of maximum tone in the main bronchus. The reduction was statistically significant both in the distal trachea (99% confidence interval for difference: 14 to 45% of maximum tone, n=8) and in the main bronchus (99% confidence interval for difference: 19 to 35% of maximum tone, n=8).

Discussion

In our guinea-pig airway model, a contractile NANC response was evoked when the smooth muscle tone was low prior to activation, and a relaxant NANC response was evoked when the tone was high prior to activation. These responses converged towards a similar level of tone, and this pattern was observed both with and without simultaneous parasympathetic activation. The parasympathetic activation produced an increase in the level of tone towards which the NANC responses converged. This parasympathetic increase was not abolished by simultaneous sympathetic activation.

The magnitude of the added contractile and relaxant NANC responses - the NANC convergence effect - constituted approximately 80% of the maximum tone in the main bronchus, thereby confirming the substantial regulatory power of the NANC responses [13, 14]. It should be noted that these powerful contractile and relaxant responses are tetrodotoxin sensitive, which confirms their neural origin [15].

Hypothetically, several factors might influence the NANC convergence effect on tone. Such factors could be the neural transmitter release, the level of smooth muscle tone prior to activation, as well as the passive tension which is applied on the smooth muscle. This does not, however, preclude the possibility that NANC neural activation produces a stabilizing effect on tone *in vivo*. There are data suggesting that forces are applied on the airway by the surrounding tissue [22, 23], and variations in tone prior to neural activation may occur due to variations in the concentration of contractile agents in the vicinity of airway smooth muscle. There are also data on human subjects which indicate that peptidergic nerve activation by inhalation of the c-fibre irritant, capsaicin, produces noncholinergic bronchconstriction when there is no induced tone prior to inhalation [24]. In contrast, capsaicin produces NANC bronchodilation when there is an increased tone prior to inhalation in human subjects [1, 3]. This is compatible with the idea that the direction and the magnitude of the NANC neural response is dependent upon the level of tone prior to stimulation.

A NANC neural mechanism, which stabilizes airway smooth muscle tone *via* a contraction or a relaxation, requires some kind of communication between the contractile and relaxant NANC response. The present study suggests that this communication is postganglionic, since it can be demonstrated by using EFS [25]. In line with this, there are data indicating that the putative relaxant NANC transmitter, VIP, inhibits the contractile NANC response at a postganglionic but prejunctional site [26]. The mechanism which determines the direction and magnitude of the NANC response appears to be independent of cyclooxygenase products, since indomethacin was continuously present during our experiments.

The parasympathetic effect on the magnitude of the NANC convergence effect was small and inconsistent. This finding was probably not due to suboptimum parasympathetic activation, because the present frequency response experiments indicated that our stimulation parameters slightly favoured parasympathetic activation rather than NANC activation. Hypothetically, tachyphylaxis of parasympathetic responses could also have contributed to our results, but separate experiments suggested no pronounced tachyphylaxis for the added parasympathetic and NANC responses, or for the NANC responses alone (data not shown).

The selective activation of the NANC neural response at different levels of tone prior to activation, adjusted tone towards a moderate level in the main bronchus. The simultaneous parasympathetic activation raised this tonus equilibrium to a high level. The parasympathetic upward shift in the tonus equilibrium was not abolished by the addition of sympathetic activation. However, the added parasympathetic and NANC responses then reached a significantly lower level of tone. In the distal trachea, the pattern was similar to that seen in the main bronchus. Although it can produce a substantial regulatory power, the NANC neural influence on smooth muscle tone might, thus, be modulated by both parasympathetic and sympathetic activation. As a result, the NANC responses, activated separately or in combination with parasympathetic or sympathetic responses, provide tools capable of stabilizing airway smooth muscle tone at low, intermediate, or high levels, with a fairly high degree of accuracy.

We used histamine in order to induce tone prior to EFS and, hypothetically, this could have reduced the parasympathetic increase of the tonus equilibrium. This is because histamine can produce an inhibitory effect on cholinergic neurotransmission in the guinea-pig isolated trachea [27]. However, this histamine-induced inhibition is primarily mediated by H3-receptors located on parasympathetic ganglia. Cholinergic responses to postganglionic activation by EFS are reduced by a maximum of 10% only [27]. It therefore appears unlikely that our use of histamine significantly affected the cholinergic, parasympathetic responses to EFS.

The sympathetic downward "shift" in the tonus equilibrium could illustrate the point of having this adrenergic and the NANC relaxant mechanisms working

side-by-side. The added regulatory power of these two mechanisms might be required to counteract severe bronchial smooth muscle contraction. In humans, the adrenergic influence on tone appears to be mediated by circulating catecholamines, rather than neurally-released ones [4, 5], but both in the guinea-pig and in man, the adrenergic smooth muscle relaxation is mediated primarily by beta$_2$-adrenoceptors [28, 29]. The sympathetic neural response can, therefore, be regarded as a model of endogenous adrenergic mechanisms in human airways. Adrenergic mechanisms may, thus, provide additional protection from severe smooth muscle contraction *via* functional interactions with the NANC and the parasympathetic neural systems.

Functional interactions between the different regulatory systems in the airways are indicated, not only by the present study, but also by others. For example, the parasympathetic neural influence on tone is facilitated by tachykinins in the guinea-pig *in vitro* [30, 31], whereas VIP inhibits the parasympathetic influence in the same species [25, 26]. Furthermore, substance P accelerates the release of acetylcholine in rabbit airways *in vitro* and *in vivo* [32].

In human as well as in guinea-pig airways, the cholinergic contractile influence on tone, mediated by parasympathetic nerves, can produce a considerable regulatory power [15]. In contrast to this, we found no significant parasympathetic influence on the magnitude of the NANC convergence effect. The level of tone towards which the NANC responses converge was also moderately increased by parasympathetic activation. It is therefore possible that the NANC responses produce so much regulatory power that an imbalance between these contractile and relaxant responses contribute to severe smooth muscle contraction. This could be *via* an increased contractile NANC response and/or *via* a reduced relaxant NANC response. If so, it would be in line with the suggested imbalance between the putative contractile and relaxant NANC transmitters, SP and VIP, in the airways of asthmatic subjects [6, 8]. The bronchoconstriction induced by the putative contractile NANC transmitter, neurokinin A, in asthmatic subjects but not in normal ones is also compatible with this idea [33]. A lack of relaxant NANC transmitter is also consistent with VIP causing bronchodilation and providing protection from histamine-induced bronchoconstriction in asthmatic subjects [34]. In this respect, the role of nitric oxide, another putative relaxant NANC transmitter in human and guinea-pig airways [35–38], is in need of further evaluation.

In conclusion, this study indicates that contractile and relaxant NANC neural responses stabilize smooth muscle tone in guinea-pig isolated airways. This stabilizing NANC effect displays a considerable magnitude and simultaneous parasympathetic activation does not change its magnitude significantly. The level of tone towards which the NANC responses converge is, however, increased by simultaneous parasympathetic activation and reduced by simultaneous sympathetic activation. The NANC stabilizing effect on tone may, thus, be modulated by both parasympathetic and sympathetic activation. In this way, the tone can be stabilized at low, intermediate, or high levels.

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