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Effects of five different airway smooth muscle relaxants on inhibitory neurotransmission in isolated guinea-pig trachea in vitro

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Abstract

Pharmacodynamic effects produced by terbutaline (10 nM), theophylline (10 μM), sodium nitroprusside (30 nM), levcromakalim (0.3 μM) or isradipine (1 nM) on frequency-dependent relaxations induced by electric field stimulation of either proximal or distal parts of isolated guinea-pig trachea were studied in vitro. Preparations were depleted for tachykinins by capsaicin, pretreated with atropine (0.1 μM) and contracted by histamine (2 μM). Drug effects were studied in preparations with combined adrenergic and inhibitory non-adrenergic non-cholinergic (NANC) innervation and in preparations with inhibitory NANC innervation either with or without additional treatment with N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM). In preparations with combined adrenergic and inhibitory NANC innervation terbutaline, sodium nitroprusside, levcromakalim and isradipine significantly reduced relaxant responses to electric field stimulation in proximal preparations, whereas distal preparations were only affected by terbutaline. In preparations with inhibitory NANC innervation without L-NAME pretreatment, terbutaline significantly enhanced relaxant responses to electric field stimulation only in distal preparations, whereas theophylline, sodium nitroprusside and levcromakalim significantly augmented responses to electric field stimulation in both proximal and distal preparations. In preparations with inhibitory NANC innervation pretreated with L-NAME, theophylline significantly inhibited relaxant responses in distal preparations, whereas sodium nitroprusside, levcromakalim and isradipine significantly augmented relaxant responses to electric field stimulation in proximal preparations. It was concluded that drugs used in the present study can modulate the effects of inhibitory autonomic and NANC neurotransmission in isolated guinea-pig trachea. Furthermore, it was shown that some variation in drug effects exists in relation to proximal and distal parts of guinea-pig trachea. © 1998 Elsevier Science B.V.

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1. Introduction

In addition to the adrenergic (sympathetic) and cholinergic (parasympathetic) innervation of airway smooth muscle, human and guinea-pig airways receive a non-adrenergic non-cholinergic (NANC) innervation consisting of an excitatory component mediating bronchoconstriction and an inhibitory component mediating bronchodilation (Ellis and Undem, 1994b; Barnes, 1993). NANC neurotransmission has been shown to exert significant regulatory influence on airway tone (Lindén, 1992) and calibre (Mackay et al., 1991). Functional imbalance between inhibitory and excitatory NANC innervation may be a part of asthma pathophysiology (Barnes, 1993). The tachykinins involved in excitatory NANC neurotransmission include substance P, neurokinin A and neurokinin B (Barnes, 1993; Ellis and Undem, 1994b), whereas transmitters involved in inhibitory NANC neurotransmission include nitric oxide (NO) and vasoactive intestinal peptide (VIP) in guinea-pig airways (Matsuzaki et al., 1980; Li and Rand, 1991) and NO in human airways (Belvisi et al., 1992).

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In guinea-pig airways, the density of adrenergic nerves markedly decreases from the proximal (laryngeal) to the distal (carinal) end of trachea and adrenergic nerves are almost absent in the bronchioles (O'Donnell et al., 1978). A similar anatomical variation is seen with regard to inhibitory NANC innervation. This innervation functionally predominates in proximal parts of guinea-pig trachea and is progressively antagonised more distally by an increasing excitatory NANC innervation (Ellis and Undem, 1990). In human airways, sparse adrenergic nerves associated with smooth muscles have been demonstrated from the level of the lobar bronchi to the terminal bronchioles (Laitinen, 1985). Prejunctional adrenergic inhibition of cholinergic neurotransmission (Barnes, 1993), rather than direct control of airway smooth muscle, seems, however, to have functional importance and thereby making inhibitory NANC neurotransmission the major, possibly the only, direct bronchodilator innervation of human airways (Richardson and Beland, 1976).

The regulatory power of NANC neurotransmission and the possible involvement in asthma pathophysiology makes pharmacological modulation of airway NANC neurotransmission of considerable interest with regard to antiasthmatic drugs. Suppressive effects against inhibitory NANC neurotransmission could, in theory, limit the usefulness of new antiasthmatic drugs, whereas enhancement of the inhibitory NANC innervation could be of value in their future use. Bronchodilators constitute an essential part of the therapeutics against asthma, and many different cellular mechanisms involved in airway smooth muscle relaxation could be the target for future antiasthmatic drugs (Barnes, 1996; Knox and Tattersfield, 1995). In addition to stimulation of β_2-adrenoceptors (e.g., terbutaline) or inhibition of cyclic nucleotide breakdown (e.g., theophylline) bronchodilator mechanisms include activation of guanylate cyclase with NO liberated from NO-donors (e.g., sodium nitroprusside), plasmalemmal hyperpolarization through opening of ATP-sensitive K^+ channels (e.g., levcromakalim) and inhibition of Ca^{2+} influx through voltage-operated Ca^{2+} channels (e.g., isradipine) (Knox and Tattersfield, 1995). Drugs acting through these cellular mechanisms do not only produce airway smooth muscle relaxation but could, in theory, interact with airway neurotransmission at both pre- and post-junctional sites.

The present study investigates the pharmacodynamic modulation of inhibitory neurotransmission produced by the highest non-relaxant concentrations of either terbutaline, theophylline, sodium nitroprusside, levcromakalim or isradipine in isolated guinea-pig trachea in vitro. Drugs were studied against effects of either combined adrenergic and inhibitory NANC neurotransmission or inhibitory NANC neurotransmission, with or without concomitant inhibition of NO synthesis. Furthermore, experiments were conducted in proximal and distal parts of trachea in order to detect any anatomically related difference in drug effects.

2. Material and methods

2.1. Tracheal preparation and measurement of contractile force

Hartley–Dunkin guinea-pig of either sex (SPF-quality; age: 3–6 weeks; weight: 225–450 g) were stunned by a sharp blow to the neck and exsanguinated. The thorax was opened and heart, lungs and full length trachea were transferred to cold (4°C) oxygenated Krebs solution. Trachea was cleaned of fat and connective tissue under a dissection microscope and separated from the rest of the preparation by cutting the main bronchi and then cut into tubular preparations comprising two cartilage segments (approximate length: 2 mm). Six preparations, three each obtained from proximal (laryngeal) and distal (carinal) trachea, respectively, were transferred to organ baths (5 ml) containing Krebs solution (37°C, pH = 7.4) continuously gassed with oxygen (95%) and carbon dioxide (5%). Tracheal preparations were mounted in precision myographs for measurement of isometric force (Nielsen-Kudsk et al., 1986) and suspended under a passive load of 0.6 g, which is optimal for development of active force. The cyclooxygenase inhibitor indomethacin (2 μM) was added to the baths and present throughout the experiments in order to prevent development of spontaneous tone and thereby assure determination of baseline. Preparations equilibrated for 100 min with frequent exchange of Krebs solution before further experimentation. Passive load was readjusted to 0.6 g if necessary. Six experiments were run in parallel and the amplified transducer signals were recorded on a six-channel recorder (Graphtec® WR3101).

2.2. Capsaicin treatment

To prevent interference from tachykinins released by excitatory NANC nerves all preparations were treated with capsaicin (1 μM) for 30 min followed by washing with Krebs solution every 10 min for 60 min (Ellis and Undem, 1990). Capsaicin selectively depletes afferent C- and Aδ-fibres for their content of tachykinins (Bevan and Szolcsanyi, 1990) and the described procedure completely desensitise tracheal preparations to subsequent addition of capsaicin (Ellis and Undem, 1994a).

2.3. Experiments

2.3.1. Neurostimulation

The myographs were equipped with electrode holders allowing precise positioning of two stainless steel electrodes on opposite sides of each tracheal preparation with an interelectrode distance of 5 mm. Electric field stimulation was performed by use of a Grass® S88 electric stimulator with square pulses (3 ms) of supramaximal strength (40 V) for 20 s. Preparations were stimulated with 1, 3, 7, 10 and 30 Hz and allowed to return to prestimula-

tion level of contraction between each stimulation. Control experiments including the neuronal Na^+ channel blocker tetrodotoxin (3 μM) were performed in order to confirm the neural origin of responses.

2.3.2. Preparations with combined adrenergic and inhibitory NANC neurotransmission

All preparations were treated with atropine (0.1 μM) to inhibit cholinergic neurotransmission and subsequently (after 5 min) contracted by histamine (2 μM). Contractions were allowed to stabilise before addition of either terbutaline (10 nM), theophylline (10 μM), sodium nitroprusside (30 nM), levcromakalim (0.3 μM) or isradipine (1 nM). These drug concentrations were chosen as the highest non-relaxant concentrations based on separate dose-response data obtained in both proximal and distal preparations pretreated as described above (data not shown). Experiments involving isradipine or sodium nitroprusside were conducted in dim light in order to prevent photodegradation of these drugs. Preparations were equilibrated further 15 min before start of electric field stimulation.

2.3.3. Preparations with inhibitory NANC neurotransmission

All preparations were treated with atropine (0.1 μM). Inhibition of adrenergic neurotransmission was accomplished by treatment with propranolol (1 μM) except in preparations selected for experiments with terbutaline. These preparations were treated with guanethidine (10 μM) during the washing period following capsaicin treatment (see above) and then treated with guanethidine (1 μM) throughout the rest of the experiment. Preparations were subsequently (after 5 min) contracted by histamine (2 μM) and drugs added before start of electric field stimulation as described above.

In order to study drug effects against the part of inhibitory NANC not mediated by nitric oxide, a group of preparations were additionally treated with N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM) to inhibit neuronal nitric oxide synthesis. L-NAME was added together with drugs as described above and incubation for 15 min was awaited before start of electric field stimulation. Control experiments in which preparations were additionally incubated with the proteolytic enzyme α-chymotrypsin (1 U/ml) in order to inhibit responses mediated by neuropeptides (mainly VIP) were also conducted.

2.4. Data analysis and statistics

All data are expressed as means $\pm$ S.E.M. The relaxation induced by electric field stimulation is expressed as percentage reduction in histamine (2 μM) induced tone prior to stimulation (cf. Section 3). Relaxation reaching baseline was taken as 100%. Response half-lives were calculated as the time required for preparations to return half-way to prestimulation levels. Frequency–relaxation curves and frequency–half-life curves were constructed and statistically compared by two-way analysis of variance (two-way ANOVA), whereas all other statistical comparisons were made by one-way analysis of variance (one-way ANOVA) with Bonferroni's multiple comparison test. GraphPad Prism® version 2.01 (GraphPad Software, USA) was used for graphical presentation of data and statistical tests. *P*-values less than 5% were considered significant.

2.5. Drugs and solutions

Indomethacin, capsaicin, atropine sulfate, DL-propranolol hydrochloride, guanethidine monosulfate, histamine dihydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME), sodium nitroprusside dihydrate, tetrodotoxin and α-chymotrypsin were obtained from Sigma-Aldrich, UK. The following drugs were kindly donated by their producers: terbutaline and theophylline (Astra-Draco, Sweden), levcromakalim (BRL 38227) (SmithKline Beecham, UK) and isradipine (Sandoz, Switzerland).

Stock solutions used were: atropine (10 mM), propranolol (10 mM), guanethidine (10 mM), histamine (10 mM), L-NAME (10 mM), sodium nitroprusside (10 mM), terbutaline (1 mM) and α-chymotrypsin (200 U/ml) in distilled water, indomethacin (8.38 mM) in 5% $NaHCO_3$, theophylline (0.1 M) in one part 0.5 M NaOH plus four parts saline 0.9%, tetrodotoxin (0.1 mM) in acetate buffer (pH = 4.5), levcromakalim (10 mM) in 70% ethanol, capsaicin (10 mM) and isradipine (1 mM) both in 96% ethanol. All stock solution were kept at -70°C until use and then further diluted with saline 0.9%. Final bath-concentrations of ethanol in experiments involving levcromakalim or isradipine were 0.021‰ and 0.00096‰, respectively. Ethanol 0.021‰ did not modulate the effects of inhibitory neurotransmission in either proximal or distal trachea (data not shown).

The composition of the Krebs solution was (in mM): NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15 and glucose 5.5.

3. Results

Histamine (2 μM) induced a monophasic and sustained contractile response in proximal and distal preparations. In proximal preparations, the sustained level of contraction was 2.70 ± 0.09 g ($n = 36$) and neither inhibition of adrenergic neurotransmission nor the additional treatment with L-NAME significantly affected this level of contraction (2.70 ± 0.10 g ($n = 44$) and 2.92 ± 0.07 g ($n = 35$), respectively). In distal preparations, the sustained level of contraction was 2.22 ± 0.08 g ($n = 32$) and this was also unaffected by inhibition of adrenergic neurotransmission and the additional treatment with L-NAME (2.29 ± 0.05 g ($n = 40$) and 2.31 ± 0.08 g ($n = 36$), respectively). How-

ever, histamine induced contractions were significantly lower in distal preparations compared to proximal preparations.

Electric field stimulation of preparations from proximal trachea with combined adrenergic and inhibitory NANC innervation resulted in phasic relaxant responses which increased frequency-dependent from $25.73 \pm 1.83\%$ ($n = 9$) at 1 Hz to $94.58 \pm 1.01\%$ ($n = 9$) at 30 Hz. Responses at 1 to 10 Hz were abolished by tetrodotoxin-treatment, whereas responses at 30 Hz were only partially blocked resulting in $49.32 \pm 8.82\%$ ($n = 4$) relaxation. Treatment with propranolol almost abolished the relaxant response at 1 Hz ($0.49 \pm 0.26\%$ ($n = 11$)) and reduced responses to all other frequencies. Additional treatment with L-NAME further decreased these responses. In preparations treated with guanethidine, either alone or in combination with L-NAME, frequency–relaxation curves were not statistically different from those obtained with propranolol ($P = 0.79$ and $P = 0.19$, respectively). Adding α-chymotrypsin (1 U/ml) to preparations treated with both propranolol and L-NAME did not further reduce the relaxant responses to electric field stimulation up to 10 Hz and only marginally reduced the relaxant response at 30 Hz. However, α-chymotrypsin (1 U/ml) totally abolished the relaxant effect of exogenously applied VIP (10 nM). Frequency–relaxation curves obtained in preparations from proximal trachea are shown in Fig. 1A.

In preparations from distal trachea with combined adrenergic and inhibitory NANC innervation electric field stimulation resulted in phasic relaxations. The relaxant responses at all frequencies studied were less that those seen in preparations from proximal trachea and increased frequency-dependent from $7.36 \pm 2.10\%$ ($n = 7$) at 1 Hz to $74.61 \pm 3.42\%$ ($n = 7$) at 30 Hz. As in proximal trachea, responses at 1 to 10 Hz showed full sensitivity to tetrodotoxin treatment, whereas stimulations at 30 Hz only were partially blocked ($30.91 \pm 4.31\%$ ($n = 6$)). Treatment with either propranolol or guanethidine abolished the relaxant response at 1 Hz and reduced responses to all other frequencies to the same extent ($P = 0.39$). Additional treatment with L-NAME resulted in only marginally further reduction in relaxant responses. α-Chymotrypsin (1 U/ml) only affected the relaxant response at 30 Hz reducing this response to a level similar to that seen in the presence of tetrodotoxin ($26.70 \pm 6.59\%$ ($n = 4$)) and abolished the relaxant effect of exogenously applied VIP (10 nM). Frequency–relaxation curves obtained in preparations from distal trachea are shown in Fig. 1B.

In both proximal and distal preparations with combined adrenergic and inhibitory NANC innervation response, half-lives slightly lengthened as stimulation frequency increased from 1 to 10 Hz and showed marked prolongation following stimulation at 30 Hz. Pretreatment with either propranolol alone or propranolol and L-NAME did not

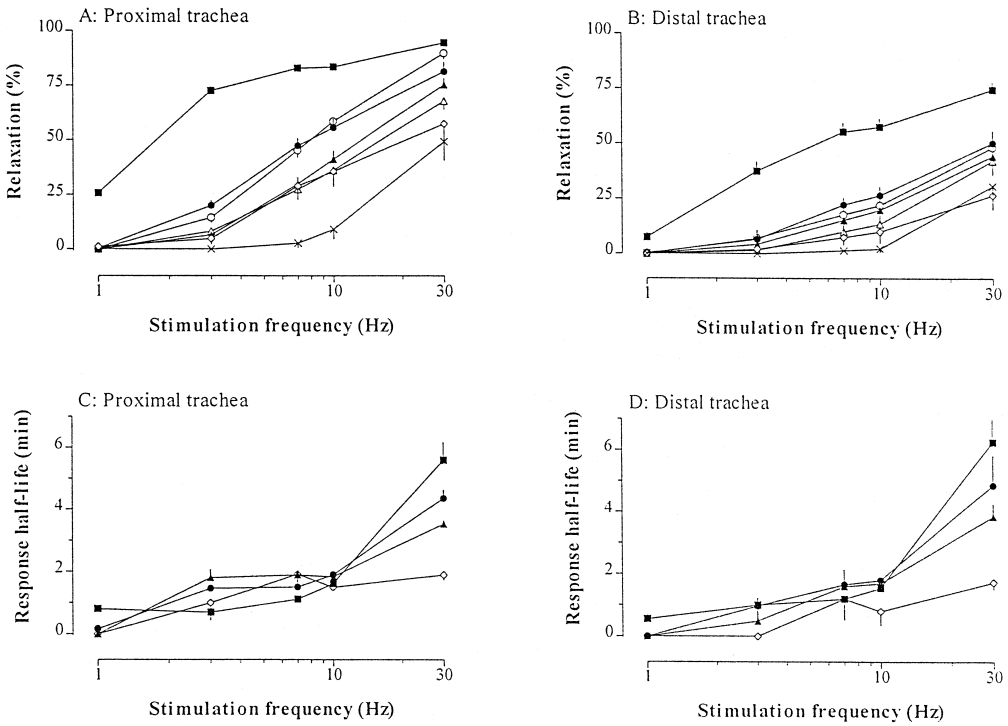

Fig. 1. Relaxant effects and response half-lives produced by electric field stimulation (40 V, 3 ms, 20 s) of either proximal (A, C) or distal (B, D) parts of isolated guinea-pig trachea depleted for tachykinins by capsaicin treatment, pretreated with atropine (0.1 μM) and contracted by histamine (2 μM) (■). Preparations were additionally treated with propranolol (1 μM) (●), propranolol (1 μM) and L-NAME (100 μM) (▲), guanethidine (1 μM) (○) or guanethidine (1 μM) and L-NAME (100 μM) (△). Preparations treated with both propranolol (1 μM) and L-NAME (100 μM) were investigated in the presence of α-chymotrypsin (1 U/ml) (◇). Control experiments with tetrodotoxin (3 μM) were also conducted (×). Error bars represent S.E.M. ($n = 4$–11).

affect half-lives in response to 1 to 10 Hz but shortened those in response to 30 Hz. However, adding α-chymotrypsin (1 U/ml) to preparations treated with both propranolol and L-NAME markedly reduced response half-lives at 30 Hz without affecting those in response to 1 to 10 Hz. Frequency–half-life curves obtained in proximal preparation are shown in Fig. 1C and those obtained in distal preparations are shown in Fig. 1D.

3.1. Inhibitory neurotransmission effects modulated by airway smooth muscle relaxants

3.1.1. Effects of terbutaline

Terbutaline (10 nM) treatment of preparations with combined adrenergic and inhibitory NANC innervation obtained from proximal trachea (Fig. 2A) resulted in reduced relaxant responses to electric field stimulation. The relaxant responses were reduced at all frequencies resulting in a significant ($P < 0.0001$) displacement of the frequency–relaxation curve. Relaxant responses in preparations with inhibitory NANC innervation with and without concomitant L-NAME treatment were unaffected. In preparations from distal trachea (Fig. 2B), terbutaline (10 nM) reduced relaxant responses in preparations with combined adrenergic and inhibitory NANC innervation and significantly ($P = 0.006$) displaced the frequency–relaxation curve. In contrast to this, terbutaline (10 nM) enhanced relaxant responses in preparations with inhibitory NANC innervation without L-NAME treatment but did not affect responses in preparations additionally treated with L-

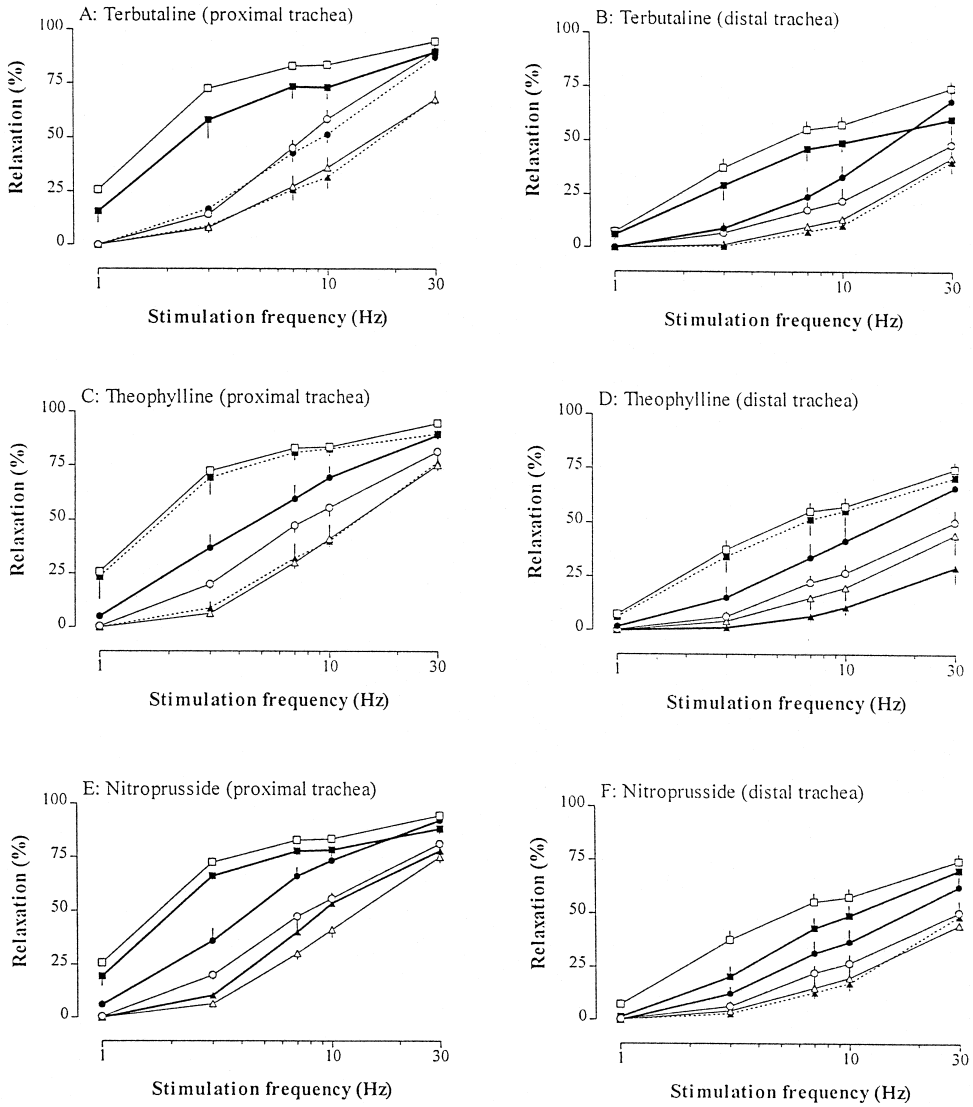

Fig. 2. Relaxant effects produced by electric field stimulation (40 V, 3 ms, 20 s) of isolated guinea-pig trachea either with (filled symbols) or without (hollow symbols) pretreatment with terbutaline (10 nM) (A, B), theophylline (10 μM) (C, D) or sodium nitroprusside (30 nM) (E, F). Preparations were depleted for tachykinins by capsaicin treatment, pretreated with atropine (0.1 μM) and contracted by histamine (2 μM) (■, □) or additionally treated with propranolol (1 μM) either with (▲, △) or without (●, ○) concomitant treatment with L-NAME (100 μM). In preparations pretreated with terbutaline, propranolol (1 μM) were substituted by guanethidine (1 μM). Hatched curves indicate non-significant effects, whereas significant modulation is indicated by dense curves. Error bars represent S.E.M. ($n = 5–11$).

Table 1
Summary of effects obtained with terbutaline (10 nM), theophylline (10 μM), sodium nitroprusside (30 nM), levcromakalim (0.3 μM) and isradipine (1 nM) on inhibitory neurotransmission in isolated guinea-pig trachea

	Innervation present in preparations					
	Adrenergic + inhibitory NANC		Inhibitory NANC		Inhibitory NANC with L-NAME	
	Proximal	Distal	Proximal	Distal	Proximal	Distal
Terbutaline	↓	↓	⇌	↑	⇌	⇌
Theophylline	⇌	⇌	↑	↑	⇌	↓
Nitroprusside	↓	↓	↑	↑	↑	⇌
Levcromakalim	↓	⇌	↑	↑	↑	⇌
Isradipine	↓	⇌	⇌	⇌	↑	⇌

Drugs were studied in preparations obtained from either proximal or distal parts of guinea-pig trachea. All preparations were desensitised to capsaicin, treated with atropine (0.1 μM) and contracted by histamine (2 μM). Drug effects were studied in preparations with either combined adrenergic and inhibitory non-adrenergic non-cholinergic (NANC) innervation or in preparations with inhibitory NANC innervation either with or without additionally treatment with N^G-nitro-L-arginine methyl ester (L-NAME) (100 μM).
↑: Facilitation of relaxant effect.
↓: Inhibition of relaxant effect.
⇌: No effect.

NAME. Terbutaline-induced enhancement increased with increasing stimulation frequency resulting in significant ($P = 0.004$) displacement of the frequency–relaxation curve without significant interaction between enhancing effect and frequency ($P = 0.129$). The results obtained with terbutaline are summarised in Table 1.

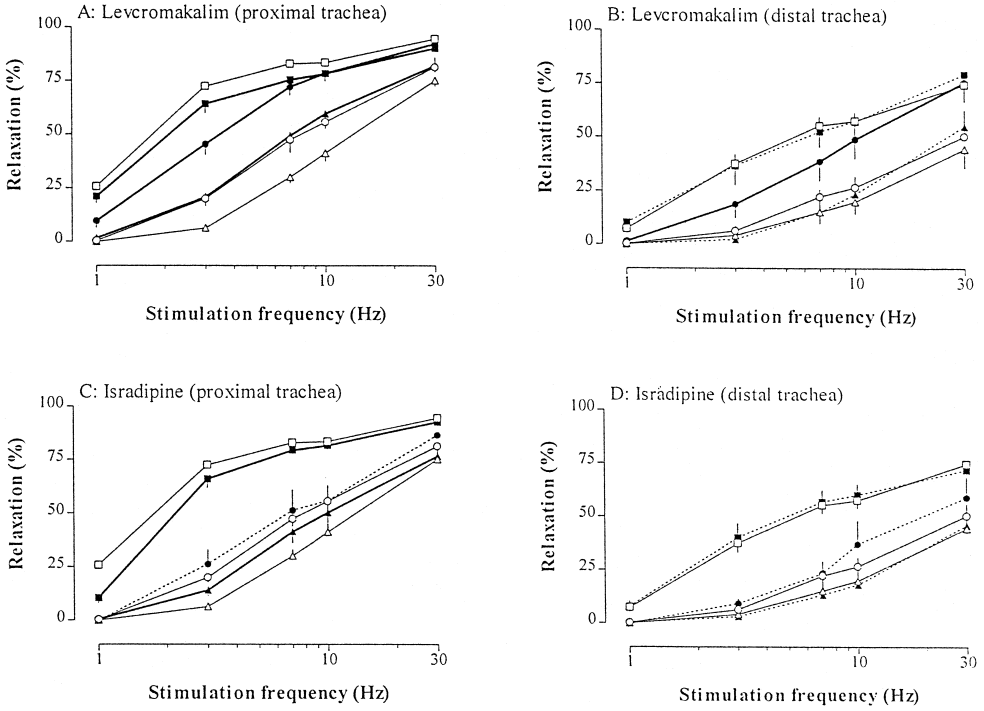

Fig. 3. Relaxant effects produced by electric field stimulation (40 V, 3 ms, 20 s) of isolated guinea-pig trachea either with (filled symbols) or without (hollow symbols) pretreatment with levcromakalim (0.3 μM) (A, B) or isradipine (1 nM) (C, D). Preparations were depleted for tachykinins by capsaicin treatment, pretreated with atropine (0.1 μM) and contracted by histamine (2 μM) (■, □) or additionally treated with propranolol (1 μM) either with (▲, △) or without (●, ○) concomitant treatment with L-NAME (100 μM). Hatched curves indicate non-significant effects, whereas significant modulation is indicated by dense curves. Error bars represent S.E.M. ($n = 5–11$).

3.1.2. Effects of theophylline

Frequency–relaxation curves obtained in preparations from proximal trachea (Fig. 2C) with combined adrenergic and inhibitory NANC neurotransmission was not significantly displaced ($P = 0.242$) by theophylline (10 μM) treatment. Theophylline (10 μM) significantly enhanced ($P < 0.001$) the relaxant responses in proximal preparations with inhibitory NANC neurotransmission without L-NAME treatment, but this was not seen in preparations with additional L-NAME treatment ($P = 0.681$). In distal preparations (Fig. 2D), effects of combined adrenergic and inhibitory NANC neurotransmission were not modulated by theophylline (10 μM) treatment ($P = 0.998$). Theophylline (10 μM) significantly enhanced the effects of inhibitory NANC neurotransmission in distal preparations without L-NAME treatment ($P = 0.001$), but on the contrary, inhibited these effects in preparations concomitantly treated with L-NAME ($P = 0.023$). Results obtained with theophylline are summarised in Table 1.

3.1.3. Effects of sodium nitroprusside

Sodium nitroprusside (30 nM) significantly reduced the effects of combined adrenergic and inhibitory NANC neurotransmission in preparations from proximal trachea ($P < 0.001$), whereas effects of inhibitory NANC neurotransmission either with or without concomitant L-NAME treatment were significantly enhanced ($P = 0.004$ and $P < 0.001$, respectively) (Fig. 2E). In distal preparations (Fig. 2F), effects of combined adrenergic and inhibitory NANC neurotransmission were inhibited ($P = 0.0003$) by sodium nitroprusside (30 nM) and effects of inhibitory NANC neurotransmission without L-NAME were enhanced ($P = 0.017$). In contrast to results obtained in preparations from proximal trachea, sodium nitroprusside (30 nM) did not modulate the effects of inhibitory NANC neurotransmission in the presence of L-NAME ($P = 0.886$). Results are summarised in Table 1.

3.1.4. Effects of levcromakalim

Treatment with levcromakalim (0.3 μM) significantly ($P = 0.0001$) inhibited the relaxant effects of combined adrenergic and inhibitory NANC neurotransmission in preparations from proximal trachea (Fig. 3A), whereas effects of inhibitory NANC neurotransmission in these preparations either without or with additional L-NAME treatment were significantly enhanced ($P < 0.001$ in both situations). In preparations from distal trachea levcromakalim (0.3 μM) enhanced the effects of inhibitory NANC neurotransmission in preparations without L-NAME treatment ($P = 0.0002$), whereas effects of combined adrenergic and inhibitory NANC neurotransmission as well as inhibitory NANC neurotransmission with concomitant L-NAME treatment were unaffected ($P = 0.822$ and $P = 0.532$, respectively) (Fig. 3B). Results obtained with levcromakalim are summarised in Table 1.

3.1.5. Effects of isradipine

Isradipine (1 nM) inhibited the effects of combined adrenergic and inhibitory NANC neurotransmission in preparations from proximal trachea ($P < 0.001$). The magnitude of inhibition decreased with increasing stimulation frequency resulting in statistically significant interaction between these two parameters ($P = 0.007$). Effects in proximal preparations with inhibitory NANC neurotransmission without additional L-NAME treatment were unaffected by isradipine (1 nM), but effects in preparations with L-NAME treatment were enhanced ($P = 0.004$) (Fig. 3C). In preparations from distal trachea, all three types of neurotransmission were unaffected by isradipine (1 nM) treatment (Fig. 3D). Results are summarised in Table 1.

4. Discussion

Many drugs, including those used in the present study (terbutaline, theophylline, sodium nitroprusside, levcromakalim and isradipine), have been shown to inhibit the effects of excitatory NANC neurotransmission (Ellis and Undem, 1994b; Nielsen-Kudsk et al., 1994), whereas studies of pharmacological modulation of inhibitory NANC neurotransmission have only been performed with a limited number of drugs. These includes K^+ channel openers (Burka et al., 1991; Cooper and Maclagan, 1991), isoenzyme-selective phosphodiesterase inhibitors (Ellis and Conanan, 1995; Fernandes et al., 1994; Rhoden and Barnes, 1990), α-adrenoceptor agonists (Thompson et al., 1990), loop-diuretics, nedocromil sodium (Verleden et al., 1994) and the neuronal N-type voltage-sensitive calcium channel blocker ω-conotoxin (Altiere et al., 1992). Inhibition of excitatory neurotransmission could be misinterpreted as an augmentation of inhibitory neurotransmission. Therefore, excitatory neurotransmission has to be abolished when studying drug effects on inhibitory neurotransmission. In the present study, tracheal preparations were subjected to tachykinin depletion by capsaicin treatment and experiments were carried out in the presence of atropine in order to eliminate the effects of excitatory neurotransmission.

The present study confirms that adrenergic (Coburn and Tomita, 1973), as well as inhibitory NANC innervation (Ellis and Undem, 1990), mainly have functional importance in the proximal parts of guinea-pig trachea. Furthermore, pretreatment of preparations with inhibitory NANC innervation with the NO synthase inhibitor L-NAME resulted in a more pronounced inhibition of relaxant responses in the proximal than in the distal trachea (cf. Fig. 1A,B). This indicates that the part of inhibitory NANC neurotransmission mediated by NO has its functional predominance in the proximal parts of guinea-pig trachea and a comparable anatomical difference with regard to function has been reported in cat airways (Takahashi et al., 1995). In both proximal and distal preparations with inhibitory

NANC innervation, the combined pretreatment with L-NAME (100 μM) and α-chymotrypsin (1 U/ml) did not eliminate relaxant responses except at 30 Hz, where responses were reduced to levels near those obtained in the presence of tetrodotoxin (cf. Fig. 1A,B). However, response half-lives were markedly prolonged at 30 Hz, and this prolongation was totally abolished following treatment with α-chymotrypsin (1 U/ml) (cf. Fig. 1C,D). These observations strongly support the assumption that VIP or related neuropeptides in guinea-pig trachea mainly are released in response to high frequency stimulation (Ellis and Conanan, 1995), as seen in cat airways and rat gastric fundus (Takahashi et al., 1995; Boeckxstaens et al., 1992). Although it is currently not resolved whether tetrodotoxin-resistant responses are neuronal or non-neuronal in origin (Ellis and Undem, 1994b), the partially tetrodotoxin-resistant responses at 30 Hz could possibly reflect direct stimulation of the tracheal smooth muscle or result from electric field stimulation induced oxidative degradation of histamine (Hulsmann et al., 1993).

This is the first study to report the effects of terbutaline and theophylline against inhibitory neurotransmission in isolated guinea-pig trachea. Stimulation of presynaptic β_2-adrenoceptors has been shown to facilitate neuronal noradrenaline release in rat trachea (Brunn et al., 1994) which would augment inhibitory neurotransmission. However, in the present study, terbutaline induced inhibition of combined adrenergic and inhibitory NANC neurotransmission without inhibitory effect on inhibitory NANC neurotransmission (cf. Table 1). This indicates a selective inhibition of adrenergic neurotransmission and could possibly be caused by terbutaline acting as a partial agonist on airway smooth muscle β_2-adrenoceptors. Another explanation could possibly be a presynaptic β_2-adrenoceptor mediated inhibition of adrenergic neurotransmission.

Isoenzyme-selective phosphodiesterase inhibitors have been shown to enhance the relaxant action of inhibitory NANC neurotransmission in guinea-pig trachea by a preferential augmentation of NO-mediated relaxations (Ellis and Conanan, 1995; Rhoden and Barnes, 1990). The present study demonstrates that the non-selective phosphodiesterase inhibitor theophylline also enhances the relaxant effect of inhibitory NANC neurotransmission in both proximal and distal preparations. Moreover, this enhancement seems also to be caused by a preferential augmentation of NO-mediated relaxations since theophylline is lacking effect in proximal preparations treated with L-NAME and has reduced relaxant effect in distal preparations treated likewise (cf. Table 1).

According to our knowledge, effects of NO-donors such as sodium nitroprusside against effects of inhibitory neurotransmission in isolated airways have not previously been reported. The observed inhibitory action against effects of combined adrenergic and inhibitory NANC neurotransmission could indicate a possible prejunctional inhibition of noradrenaline release. However, the NO-donor SIN-1 (3-morpholinosydnonimine-*N*-ethylcarbamide) has been shown to be without effect on noradrenaline release from rat tail artery (Bucher et al., 1992) and equivalent measurements of noradrenaline release from guinea-pig trachea are at the moment lacking. On the contrary, sodium nitroprusside induced enhancement of inhibitory NANC neurotransmission effects in both proximal and distal preparations could be caused by a postjunctional additive interaction between sodium nitroprusside and NO liberated from inhibitory NANC nerves.

Potassium channel openers such as levcromakalim have been shown to inhibit excitatory NANC neurotransmission preferentially at a prejunctional site (Ellis and Undem, 1994b; Nielsen-Kudsk et al., 1994) and a similar effect on inhibitory neurotransmission could be expected. However, cromakalim has been reported either to be without effect against sympathetic (Pendry and Maclagan, 1991) and inhibitory NANC induced relaxations (Burka et al., 1991) or to produce a slight augmentation of inhibitory NANC induced relaxations (Cooper and Maclagan, 1991). In our study levcromakalim inhibited effects of combined adrenergic and inhibitory NANC neurotransmission and only in preparations from proximal trachea. In contrast to this, effects of inhibitory NANC neurotransmission were markedly enhanced in both proximal and distal preparations (cf. Table 1). We have previously reported an over-additive interaction between the NO-donor sodium nitroprusside and cromakalim with regard to airway smooth muscle relaxation (Thirstrup et al., 1997) and a similar synergism between neuronal liberated NO and levcromakalim-induced potassium channel opening could explain our results and also the results of Cooper and Maclagan (1991).

The lack of effect of isradipine in distal preparations and the limited effect in proximal preparations (cf. Table 1) supports the view that only N-type and not dihydropyridine sensitive L-type voltage-sensitive calcium channels are involved in regulation of autonomic and NANC neurotransmission in guinea-pig airways (Altiere et al., 1992).

The present study has shown that the airway smooth muscle relaxants terbutaline, theophylline, sodium nitroprusside, levcromakalim and isradipine modulate inhibitory autonomic and inhibitory NANC neurotransmission in isolated guinea-pig trachea. These drugs either inhibited or were without effect against relaxant responses in preparations with combined adrenergic and inhibitory NANC innervation. When adrenergic innervation was abolished, these drugs, however, either facilitated or were without effect against relaxant responses (cf. Table 1). The study has furthermore revealed some variation in drug effects between anatomically proximal and distal parts of guinea-pig trachea. Future in vivo studies are needed to clarify the clinical importance of bronchodilator-induced modulation of NANC neurotransmission with regard to the regulation of airway tone and calibre.

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