Eur Respir J 1997; 10: 314–321
DOI: 10.1183/09031936.97.10020314
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European Respiratory Journal
ISSN 0903 - 1936

L-NAME-sensitive and -insensitive nonadrenergic noncholinergic relaxation of cat airway *in vivo* and *in vitro*

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ABSTRACT: The neurotransmitters responsible for neurogenic airway relaxation are still unknown. We investigated the effects of N$^{\omega}$-nitro-L-arginine methylester (L-NAME) on nonadrenergic and noncholinergic (NANC) relaxation evoked by electrical stimulation of vagus nerves *in vivo* and *in vitro* in cat.

To that end, we measured pulmonary resistance during vagal nerve stimulation (VS) *in vivo*, and isometric tension of small bronchi (1–3 mm outer diameter) during electrical field stimulation (EFS) *in vitro*. During infusion of 5-hydroxytryptamine (5-HT), VS transiently decreased total pulmonary resistance in the presence of atropine and propranolol, with peak relaxation at several seconds after the VS and a gradual return to baseline within 2–3 min. L-NAME abolished the initial peak relaxation and reduced the peak amplitude, but did not affect the duration of the NANC relaxation. In small bronchi obtained from control cats, EFS evoked a biphasic NANC relaxation, comprising an initial fast component followed by a second slow component, and L-NAME (10^{-5} M) selectively abolished the first component without affecting the second. Whilst in the small bronchi obtained from L-NAME pretreated cats, EFS elicited only the slow component of NANC relaxation, which was insensitive to L-NAME but sensitive to tetrodotoxin.

These results indicate that nonadrenergic noncholinergic relaxation induced by vagal nerve stimulation during infusion of 5-hydroxytryptamine can be classified into two components, and that at least two neurotransmitters, including nitric oxide, are involved in the relaxation.

Eur Respir J 1997; 10: 314–321.

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Keywords: Autoregulation
excitatory junction potential
lung resistance
nonadrenergic noncholinergic relaxation
N$^{\omega}$-nitro-L-arginine methylester

Received: December 8 1995
Accepted after revision November 15 1996

This work was supported in part by grants from The Mitsubishi Foundation and The Ministry of Education, Science and Culture, Japan.

Neurally-mediated relaxation of airway smooth muscle in various animal species, including man, is largely nonadrenergic and noncholinergic (NANC) (see, for example, [1]). Although the neurotransmitter(s) responsible for NANC relaxation in the airways have not been conclusively identified, nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) have emerged as strong candidates [2–7].

In cat airway, we have shown that NANC relaxation can be classified into two components by thresholds for activation or by sensitivity to N$^{\omega}$-nitro-L-arginine methylester (L-NAME), and that the pattern of L-NAME-sensitive and -insensitive components differs in central and peripheral airways [6, 7]. Specifically, electrical field stimulation (EFS) elicited monophasic relaxation in the trachea, confirming previous observations [5, 8], but biphasic NANC relaxations, comprising an initial fast component followed by a second slow component in the bronchioles. L-NAME selectively abolished the first component of NANC relaxation without affecting the second in bronchioles, whilst, in the trachea, L-NAME completely suppressed the monophasic NANC relaxation after single or short repetitions (<5) of 1 ms pulse stimuli; however, after more stimuli (>10) of 1 or 4 ms duration, suppression of NANC relaxation was incomplete (to 40–50% of the control value) [7]. VIP antagonists partially suppressed the L-NAME-resistant NANC relaxation. These results indicate that at least two neurotransmitters, possibly NO or NO-containing compounds and VIP, are involved in NANC relaxation of cat airways. However, these experiments were performed using isolated bronchial tissue, and, therefore, it is important to observe the effects of vagal stimulation in the presence or absence of L-NAME on total pulmonary resistance *in vivo*.

We have, therefore, examined changes in total pulmonary resistance (R_L) during electrical stimulation of the cervical vagus nerves before and after treatment with L-NAME, during infusion of 5-hydroxytryptamine (5-HT). After these experiments, we excised bronchial tissues from the animals and performed *in vitro* experiments.

Methods

Measurement of pulmonary resistance

Twelve cats of either sex, weighing 2.5–4.0 kg, were used in this study. The animals were anaesthetized with

pentobarbital sodium (50 mg·kg^{-1} *i.m.*), and ventilated with a respirator (Harvard Apparatus Model 665, South Natick, MA, USA) through a tracheostomy tube at a tidal volume of 10 mL·kg^{-1} and a respiratory rate of 30 breaths·min^{-1}.

The airflow signal (V') was measured by a connected Fleisch pneumotachograph and a differential pressure transducer (Nihon Kohden TV-142T, Tokyo, Japan). To measure pleural pressure, a catheter was inserted through the sixth intercostal space into the pleural cavity with the cat in the supine position, and was sutured in place after the introduction of 3–5 mL of air. Transpulmonary pressure (P_{tp}) was measured by a differential pressure transducer (Nihon Kohden, TP-603T), with one limb attached to the intrapleural catheter and the other to a catheter in the tracheal tube. R_L was calculated according to the method of AMDUR and MEAD [9].

A catheter was inserted into a carotid artery for the measurement of blood pressure by an electric manometer (Nihon Kohden LPU-0.1), and another catheter was inserted into a jugular vein for the administration of drugs.

Bilateral cervical vagosympathetic nerves were exposed and separated into two components, and the bipolar platinum electrodes were placed on both vagal components to stimulate the intact nerves. Repetitive stimulation (10–400 pulses) was applied at a constant voltage (30 V), pulse duration (1 and 4 ms), and impulse frequency (20 Hz).

To observe NANC relaxation, vagal nerve stimulation (VS) was applied during infusion of 5-HT (20–80 μg·kg^{-1}·min^{-1}) in the presence of atropine and propranolol (2 mg·kg^{-1} each) in five cats. A bolus injection (8 mg·kg^{-1}) of L-NAME was then administered intravenously, followed by a continuous infusion (2 mg·kg^{-1}·min^{-1}). L-NAME was dissolved in 0.9% NaCl and infused contin-uously for 45 min. In the previous studies, several doses of L-NAME were used to inhibit NO synthesis. We chose 100 mg·kg^{-1} of L-NAME, because in cats it was considered to be sufficient to inhibit synthesis of NO [10–12]. This dose of L-NAME was divided into initial and continuous doses to avoid acute effects on the cardiovascular system. Fifteen minutes after discontinuation of L-NAME infusion, the effects of VS on NANC relaxation were again tested. To determine whether the effect of L-NAME was due to the inhibition of NO synthesis, we examined the effect of the inactive enantiomer N$^{\omega}$-nitro-D-arginine methylester (D-NAME) (the same dose as L-NAME) on R_L in five cats.

In another four cats, 270 mg·kg^{-1} of L-NAME was injected intramuscularly 4 h before the experiments. The effects of VS on the NANC relaxation were then observed, and the results were compared with those obtained from control animals.

Furthermore, to observe the effects of L-NAME on cholinergic bronchoconstriction, the increase in R_L evoked by VS was observed in the presence or absence of L-NAME in three cats.

Measurement of isometric tension of the bronchi

Segments of whole pulmonary lobes were quickly resected from the main bronchus after the measurement of R_L of the L-NAME pretreated or control cats. Mainly bronchi were used in the present experiments; they were classified into two categories, namely segmental branches of lobar bronchi (3–5 mm outer diameter (OD)) and small bronchi (1–3 mm OD). Since the branching pattern in the cat airway is not symmetrical and regular as it is in humans [13], but rather similar to that of dog [14], the diameter of bronchi does not necessarily correlate with their branching order. The diameters of segmental bronchi or lobar bronchi in each lobe ranged 3–5 mm in inner diameter, and bronchioles (>1 mm) can be easily identified by the lack of cartilage on microscopic observation. Bronchi with inner diameter of 1–3 mm were classified as small bronchi. The small airways (about 1–5 mm in diameter) were carefully excised from the lung tissue under microscopic observation, and lung parenchyma and pulmonary vessels running along bronchiolar branches were carefully removed under microscopic observation. Small bronchi were used in the present experiments, since biphasic NANC relaxations could easily be evoked by EFS in the tissue [7]. Airway epithelium was carefully removed as much as possible by mechanical rubbing, according to a method described previously, since it is known that EFS stimulates airway epithelial cells to release factor(s) that induce the relaxation of dog bronchioles in the presence of indomethacin, atropine, guanethidine, and were precontracted with 5-HT (10^{-5} M) [15]. The preparation was bathed in a modified Krebs solution of the following ionic concentrations (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5, and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2 and its pH was 7.3–7.4.

The effects of L-NAME (10^{-5} M) on the amplitude of contractions evoked by stepwise increase in the stimulus number at high frequency (20 Hz) were observed, since it is known that exogenous or endogenous NO has a prejunctional action in inhibiting excitatory neuroeffector transmission in the cat airway [3, 6]. Cat airway smooth muscle cells are innervated both by cholinergic and adrenergic nerves [16], and norepinephrine released from sympathetic nerves activates prejunctional β-adrenoceptors to inhibit cholinergic excitatory neuroeffector transmission [17]. Therefore, the experiments were carried out in the presence of guanethidine (10^{-6} M).

To measure the mechanical responses of ring preparations of bronchi, the preparations were hooked horizontally in a 1 mL organ bath (through which the test solution flowed continuously, at a rate of 2 mL·min^{-1}), by a pair of right-angled fine needles that were reduced in diameter by electrolysis to about 50 μm, as observed under a microscope. One needle was fixed to the wall of the chamber. The other needle was connected to a manipulator at one end and at the other end to an isometric tension transducer, through a 1 mm width slit made on the other wall of the chamber. EFS was applied through a pair of Ag-AgCl plates fixed to opposite sides of the inner surface of the chamber, so that current pulses would pass transversely across the ring preparations of the bronchi and bronchioles.

Drugs

The following drugs were used: isoprenaline-bitartrate (Nakarai Chemicals, Kyoto, Japan), N$^{\omega}$-nitro-L-arginine

methylester (L-NAME), N$^{\omega}$-nitro-D-arginine methylester (D-NAME), 5-hydroxytryptamine hydrochloride (5-HT), L-isoprenaline hydrochloride and acetylcholine chloride (Sigma, St Louis, USA), guanethidine monosulphate (Tokyo Kasei, Tokyo, Japan), atropine sulphate (Daiichi, Tokyo, Japan), propranolol, pentobarbital sodium and tetrodotoxin (Sankyo, Tokyo, Japan). The drugs were added to the perfusing solution. Concentrations of drugs in the text refer to salts.

Statistics

Results (amplitude of muscle relaxations and resting membrane potential) are expressed as mean ±SD *(in vitro* experiments) or SE *(in vivo* experiments) and were analysed for statistical significance by Student's t-test. Significance was indicated when p-value was less than 0.05.

Results

Effects of L-NAME on RL of the cat

Vagal nerve stimulation during infusion of 5-HT caused a decrease in R_L This relaxation was due to activation of NANC inhibitory nerves, since a decrease in R_L was induced after pretreatment of the cat with atropine and propranolol.

Repetitive stimulations for 1 and 4 ms pulse duration at 20 Hz were applied to the vagus nerve to compare the results with those obtained *in vitro* [7]. Figure 1a–c (left column) shows an example of a decrease in P_{tp} (NANC relaxation) induced by stimulation of vagus nerves in a control animal, when 10, 20 and 30 stimuli of 1 ms pulse duration at 20 Hz was applied. When 10 stimuli of 1 ms pulse duration were applied, the change in P_{tp} was transient, with peak relaxation at 6 s after the VS and a gradual return to baseline within 2–3 min. However, when VS of 1 ms pulse duration and more than 20 repetitive stimuli at 20 Hz was applied, the change in P_{tp} rose to a plateau phase lasting for 30–60 s, starting 6–15 s after the application of VS and then returning to the baseline within 3–5 min.

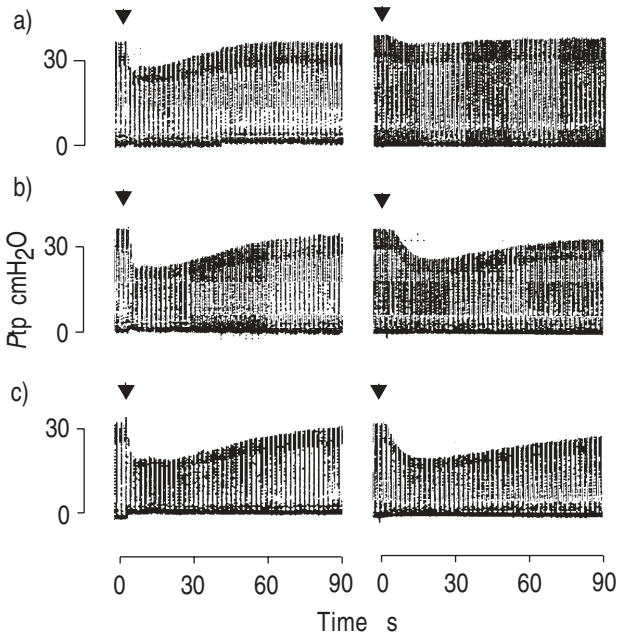

Fig. 1. – An example of changes in transpulmonary pressure (P_{tp}) induced by electrical vagal nerve stimulation (VS) of 1 ms pulse duration: a) 10; b) 20; c) 30 stimuli at 20 Hz. The left column represents results in the control condition, and the right column represents those after treatment with N$^{\omega}$-nitro-L-arginine methylester (L-NAME) (8 mg·kg^{-1} plus 2 mg·kg^{-1}·min^{-1}).

The effects of VS on the P_{tp} was then observed after infusion with L-NAME (8 mg·kg^{-1} + 2 mg·kg^{-1}·min^{-1}) (see Methods) into the cats. As shown in figure 1a–c (right column), in animals treated with L-NAME, VS (10, 20 and 30 stimuli of 1 ms pulse duration) did not evoke the initial peak relaxation, but rather a slow decrease in P_{tp}, and the maximum reduction in P_{tp} was observed 30–60 s after VS application. Thus, injection of L-NAME abolished the initial peak relaxation and reduced the peak amplitude, but did not affect the duration of the NANC relaxation induced by VS. Similar experiments were repeated with 4 ms pulse duration. Figure 2a-c show the mean change in R_L before and after the treatment with L-NAME obtained with five cats, using 1 or 4 ms pulse duration. To estimate the time course and amplitude of L-NAME sensitive change in R_L, the L-NAME-insensitive component was subtracted from the change in R_L of control condition (fig. 3a-c).

By contrast, D-NAME had no effect on airway response evoked by VS, suggesting that the effect of L-NAME was due to the inhibition of NO synthesis (fig. 4).

Similar experiments were performed separately using another four cats, pretreated with intramuscular injection of 270 mg·kg^{-1} L-NAME 4 h before the experiments, and compared the results with those of animals without L-NAME (control cats). VS induced an initial peak relaxation in R_L after several seconds, whereas in L-NAME treated cats VS did not evoke the initial peak relaxation but rather a slow and gradual NANC relaxation (data not shown). The whole lung was then excised to perform the *in vitro* experiments with trachea and bronchi.

Effects of EFS on bronchial muscle tone prepared from L-NAME treated cats

To determine the mechanisms involved in the effects of L-NAME on R_L, we observed the effects of EFS on the muscle tone of bronchial tissues prepared from control or L-NAME pretreated cats. EFS (repetitive stimuli of 4 ms pulse duration at 20 Hz) applied during contraction evoked by 5-HT (10^{-5} M) in the presence of atropine and guanethidine (10^{-6} M each) produced biphasic NANC relaxation in bronchial tissues prepared from control cats. Specifically, EFS evoked an initial peak relaxation a few seconds after the application of EFS, which was followed by a second component of NANC relaxation with a slower time course. Application of L-NAME (10^{-5} M) selectively abolished the initial peak relaxation without affecting the second component (data not shown), confirming the previous observations [7]. Thus, in the presence of L-NAME, EFS evoked a

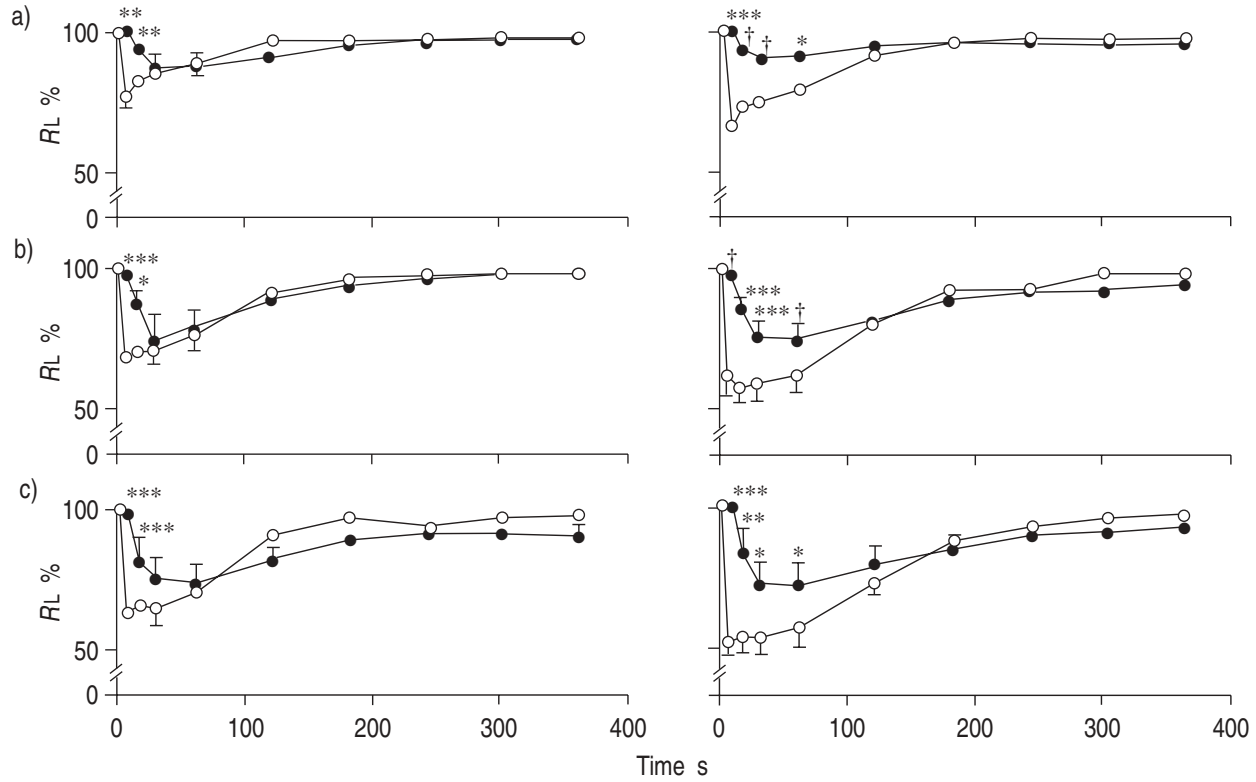

Fig. 2. – Change in total pulmonary resistance (R_L) induced by electrical vagal nerve stimulation of 1 ms (left column), or 4 ms (right column) pulse duration: a) 10; b) 20; or c) 30 stimuli, before (○) and after (●) the treatment with N^{ω}-nitro-L-arginine methylester (L-NAME) (n=5). Animals were treated with atropine and propranolol. Values are presented as mean±SD. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; †: $p<0.005$, when values are compared before and after treatment with L-NAME using Student's paired t-test.

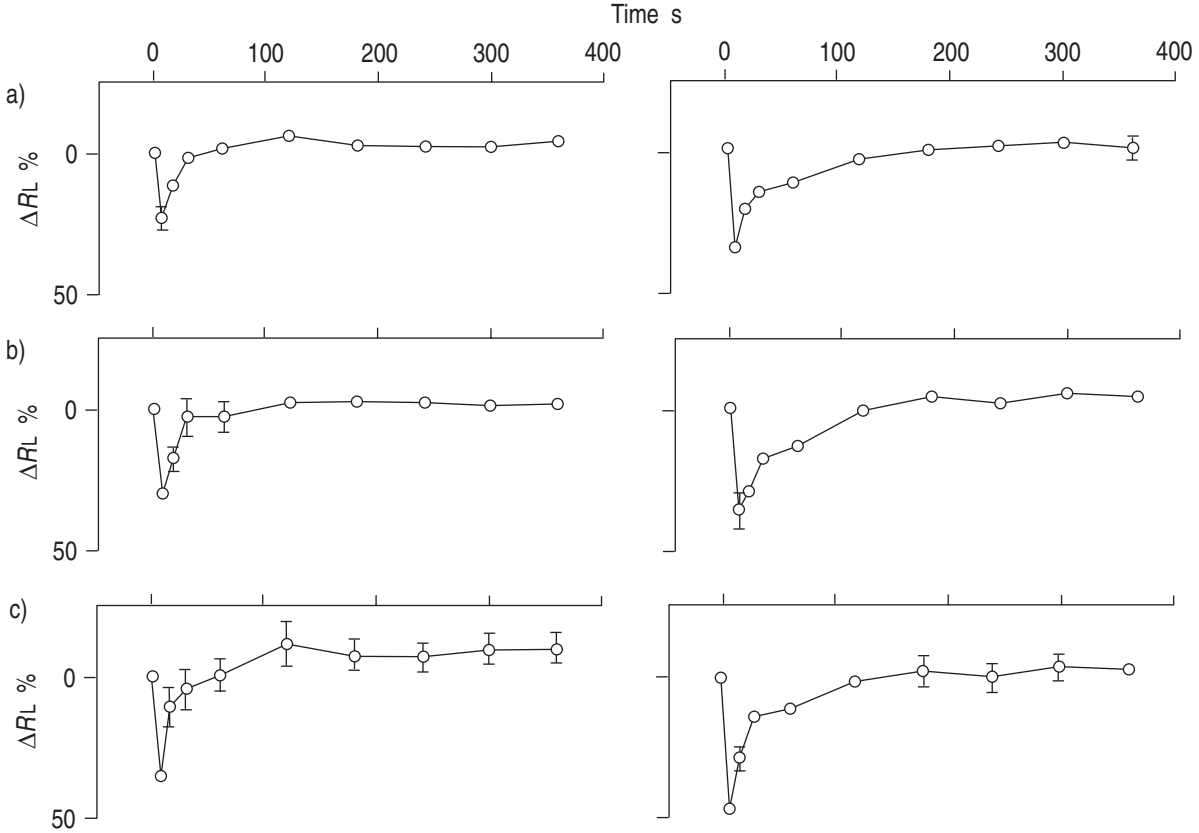

Fig. 3. – Amplitudes of N^{ω}-nitro-L-arginine methylester (L-NAME)-sensitive changes in total pulmonary resistance (R_L). The R_L after the treatment with L-NAME was subtracted from the control R_L. An electrical vagal nerve stimulation (VS) was applied of 1 ms pulse duration (left column); or 4 ms pulse duration (right column): a) 10; b) 20; or c) 30 stimuli at 20 Hz.

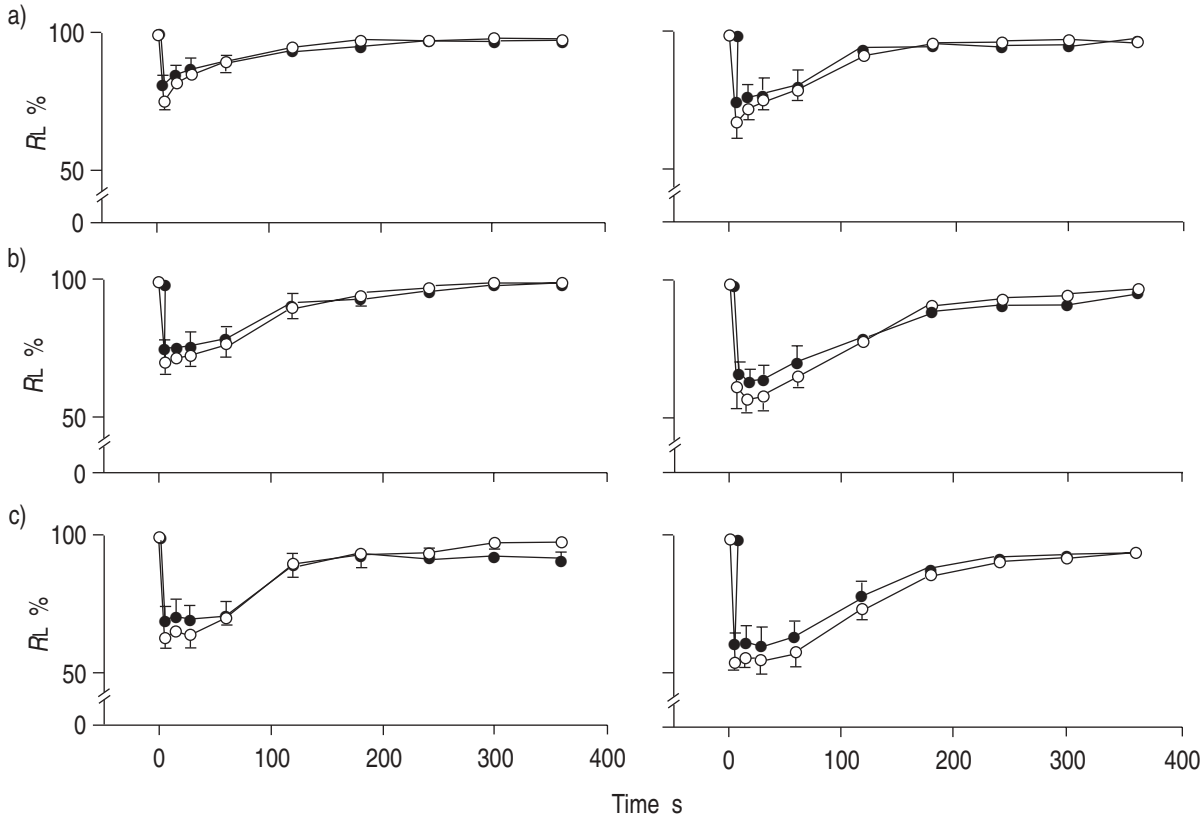

Fig. 4. – The effects of Nω-D-arginine methylester (D-NAME) on changes of total pulmonary resistance (R_L) induced by vagal stimulation (VS). D-NAME had no effect on airway response evoked by vagal stimulation (n=5), before (○) and after (●) D-NAME treatment. Electrical vagal stimulation was applied for 1 ms pulse duration (left column); or 4 ms pulse duration (right column): a) 10; b) 20; or c) 30 stimuli at 20 Hz. Values are presented as mean±SE.

relatively slow and gradual NANC relaxation. In contrast, in the bronchial tissue prepared from L-NAME pretreated cat, EFS did not evoke the initial fast NANC relaxation but only the slow and gradual, second component. Treatment of the tissue with L-NAME (10^{-5} M) did not affect the amplitude or duration of the slow and gradual NANC relaxation, but tetrodotoxin (10^{-7} M) completely abolished it (data not shown).

Effects of L-NAME on EFS-induced constriction of the cat airway in vivo *and* in vitro

Figure 5 shows the relationship between the number of stimuli at 20 Hz and relative tension in the presence or absence of L-NAME (10^{-5} M). L-NAME (10^{-5} and 10^{-4} M) significantly enhanced the amplitude of contraction evoked by repetitively applied EFS.

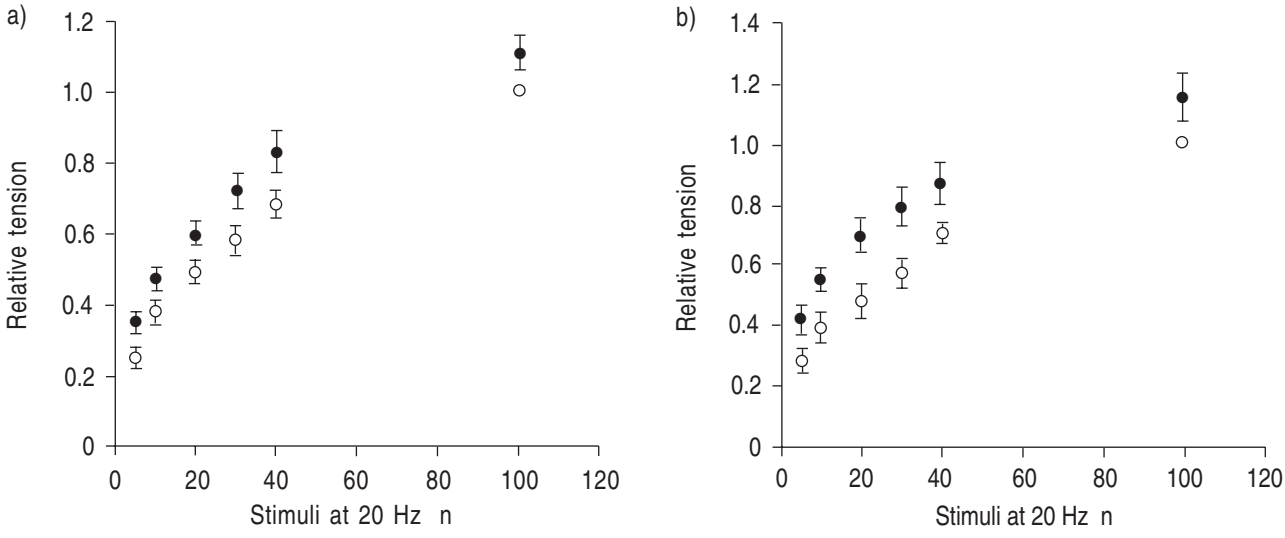

Fig. 5. – Effects of Nω-L-arginine methylester (L-NAME) at: a) 10^{-5} M; and b) 10^{-4} M, on the amplitude of contractions of cat trachea evoked by repetitive stimuli (10–100 stimuli) at 20 Hz in the presence of guanethidine. Values are presented as mean±SD. —○— : in the absence of L-NAME (control);—●—: in the presence of L-NAME.

To examine the mechanisms involved in the accelerating effects of L-NAME on the summation of the contractions, the excitatory junction potential (EJP) was recorded using a microelectrode method in tracheal tissues prepared from control or L-NAME pretreated cats. In parallel to the summation observed in the amplitude of the contractions, the amplitude of the EJPs also showed summation when repetitive stimuli at high frequency were applied, and pretreatment of the cats with L-NAME (10^{-5} M) greatly enhanced the summation (fig. 6).

To elucidate the effects of L-NAME on airway constriction evoked by VS *in vivo*, the change in R_L was also measured before and during application of L-NAME in the presence of propranolol. In this series of experiments, a VS of 1 ms pulse duration was applied for 10 s at different frequencies (10, 20 and 30 Hz), since short repetitive stimuli (10–30 stimuli at 20 Hz) did not evoke significant change in R_L. VS caused a marked increase in R_L, which was transient with a reversion to baseline within 30 s. Treatment of the cats with L-NAME did not affect the amplitude of airway constriction (fig. 7).

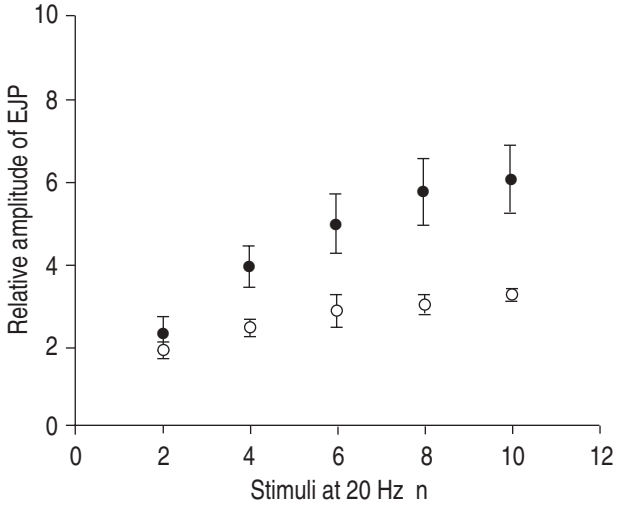

Fig. 6. – Effects of pretreatment of cat with N$^{\omega}$-nitro-L-arginine methylester (L-NAME) on the amplitude of excitatory junction potential (EJP) evoked by repetitive stimuli (2–10 stimuli) at 20 Hz in the trachea. In the trachea excised from L-NAME pretreated cat, summation of EJP amplitude was markedly enhanced. —○—: in absence of L-NAME pretreated (control); —●— : L-NAME pretreated. Values are presented as mean±SD.

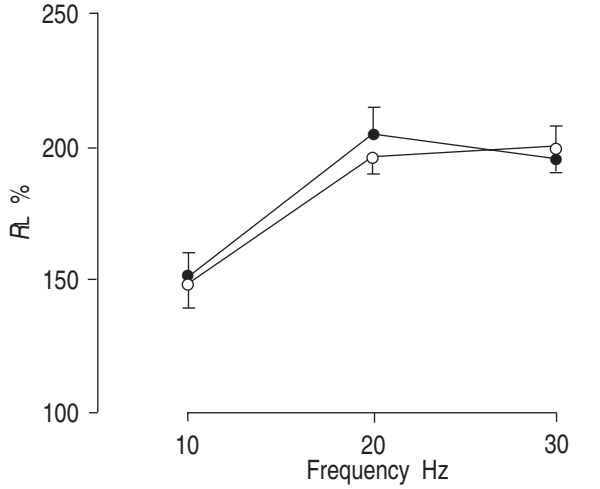

Fig. 7. – Changes in total pulmonary resistance (R_L) induced by electrical vagal nerve stimulation (VS) before (—○—) and after the treatment (—●—) with L-NAME in the presence of propranolol (n=3).

Discussion

The results of this study clearly indicate that the decrease in R_L induced by VS during infusion of 5-HT in animals treated with propranolol and atropine, can be classified into two components, L-NAME-sensitive and L-NAME-insensitive, which also have different time courses. At present, it is not technically feasible to speculate about the precise time course of the L-NAME-sensitive component, since specific inhibitors of the L-NAME-insensitive component are not available [7]. However, the time course of L-NAME-sensitive change in R_L can be estimated by subtracting the L-NAME-insensitive component from the control change in R_L. The L-NAME-sensitive change in R_L showed its peak relaxation within several seconds after the application of EFS and lasted between 6 and 60 s when a 1 ms pulse duration was employed. On the other hand, the L-NAME-insensitive change in R_L occurred gradually, reached a peak relaxation with a longer latency (30–60 s) and lasted for 2–6 min.

These observations parallel those obtained with isolated bronchial tissues in the presence of L-NAME ([7], and present observations). Namely, EFS evoked an initial peak NANC relaxation (mean±SD) 7.4±2.2 s (n=23) after the application of EFS, which was followed by a second component of NANC relaxation with a slower time course. The mean time to peak relaxation of the second component after EFS, measured in the presence of L-NAME (10^{-5} M), was 77.6±20.3 s [7]. The roles of the two components in the change in R_L may differ, especially in the peripheral airways, namely one inducing fast relaxation with short delay after the activation of NANC nerves and the other evoking long-lasting relaxation with relatively long delay. Thus, it seems that the slow component may be important for long-lasting relaxation in the peripheral airway. Thus, data obtained from the smaller airways *in vitro* is more representative of the *in vivo* state, *i.e.* the measurements *in vitro* may be more useful to observe changes in the smaller airways.

The initial fast decrease in R_L was suppressed completely by L-NAME, and, therefore, it is reasonable to conclude that the neurotransmitter responsible for this component is NO or a NO-containing compound, since L-NAME is a potent and specific inhibitor of NO-synthase (NOS) [18].

The neurotransmitters responsible for the second component of the change in R_L have yet to be determined. Immunofluorescence techniques have revealed the presence of VIP-immunoreactive nerve fibres in airway smooth muscle layers in various animal species, including man [19, 20]. Furthermore, VIP is released during EFS of guinea-pig trachea, and a correlation exists between the amount of VIP released and the degree of relaxation induced by EFS [21]. Incubation with anti-VIP-antiserum or immunization of cats to VIP reduces the tracheal relaxation in response to EFS [8, 21]. These data, taken together, suggest that VIP is one candidate for the neurotransmitter responsible for the second component of NANC relaxation in the airway. However, *in*

vitro experiments have indicated that the amplitude of the L-NAME insensitive component of NANC relaxation was only partially suppressed by VIP-antagonists or α-chymotrypsin [7], indicating that unknown neurotransmitter(s) other than VIP may be involved in L-NAME insensitive relaxation. The substantial remainder of α-chymotrypsin- and VIP-antagonist-resistant and L-NAME-insensitive NANC relaxation was suppressed by tetrodotoxin. Furthermore, the overall conclusion of earlier works concerning the effects of α-chymotrypsin on exogenously applied VIP or NANC relaxation, seems to be that VIP is not involved in the NANC relaxation [22]. These observations may also indicate that neurotransmitters other than VIP and NO are involved in the NANC inhibitory neurotransmission in the airway.

The results of the present study also indicate that L-NAME increases the amplitude of contractions evoked by repetitive EFS *in vitro*. L-NAME also increases the summation of EJPs evoked by repetitively applied EFS in the presence of guanethidine, thereby indicating that L-NAME-induced enhancement of the contraction is due to the facilitatory effects of L-NAME on excitatory-neuroeffector transmission, presumably by enhancement of transmitter release [6]. However, it was reported that inhibition of NO synthesis had no effect on acetylcholine (ACh) release in the human and guinea-pig airway [23, 24]. However, in contrast, SEKIZAWA *et al*. [25] had shown that NO synthesis inhibitor, N^G-monomethyl-L-arginine (L-NMMA), significantly enhances the ACh release from the vagus nerve terminal evoked by EFS in the guinea-pig trachea. Thus, it seems plausible to postulate that NO, when released from nerve terminals, may directly relax airway muscle through pharmacomechanical coupling, and at the same time inhibit the release of ACh from the vagus nerve terminal, thus playing a "double braking" role in bronchoconstriction [6, 7, 26]. However, L-NAME did not affect the increase of R_L induced by VS *in vivo*, although pretreatment of the cats, with L-NAME greatly enhanced airway responsiveness to inhaled 5-HT in the presence of propranolol and atropine [27]. The reasons for these discrepancies between *in vivo* and *in vitro* observations are unknown. However, it is known that in the airway, ACh release from the vagus nerve terminals is regulated in many ways by ACh itself, norepinephrine released from the postganglionic sympathetic nerve fibres, endogenous prostaglandins or VIP [16]. Thus, it may be that the treatment of the cats with L-NAME only was not enough to suppress these braking systems *in vivo*.

The clinical importance of the present findings in the pathophysiology of airway diseases is uncertain. In human airways, no evidence has been found for adrenergic innervation, and, therefore, the inhibitory NANC (i-NANC) nervous system has been reported to be a principal inhibitor of smooth muscle [28]. Recent *in vitro* experiments with human bronchial tissue have indicated that L-NAME produced a concentration-dependent inhibition of i-NANC relaxation, producing almost complete inhibition at low stimulus frequency of EFS [3], which is in sharp contrast to the present findings, that L-NAME only partially inhibits NANC relaxation in the cat airway at high stimulus frequency. VIP-immunoreactive nerve fibres are present in airway smooth muscle layers in man [20], and, furthermore, it has been reported that in asthmatic patients there is a loss of VIP from pulmonary nerve fibres and that the loss may diminish neurologically-mediated bronchodilation [29]. Inhibitory-NANC relaxation has also been demonstrated *in vivo* in healthy subjects, which can be activated by inhalation of capsaicin [30, 31] or sulphur dioxide [32], or by laryngeal stimulation [33]. Inhibition of the i-NANC system by hexamethonium or vagotomy induces airway hyperresponsiveness in cats, suggesting a possible role of i-NANC in the genesis of bronchial asthma [34, 35]. In asthmatic subjects, it has been suggested that the i-NANC system may play a crucial role in nocturnal asthma, based upon the observation that i-NANC relaxation was reduced in the early morning [36]. However, other investigators have found no differences in the amplitude of i-NANC relaxation between healthy and asthmatic subjects, and have concluded that the i-NANC system was unlikely to be important in the pathogenesis of asthma [37]. Since there are too few *in vivo* and *in vitro* findings in human airway to clarify the roles of NOS inhibitor-sensitive and -insensitive relaxation of the i-NANC nervous system in the genesis of airway hyperresponsiveness, further investigations are warranted.

From the present findings, we conclude that the decreasing response in total pulmonary resistance induced by vagal nerve stimulation in the cat airway can be classified into N^{ω}-nitro-L-arginine methylester-sensitive and -insensitive components, and that at least two neurotransmitters, possibly nitric oxide or nitric oxide-containing compounds and a transmitter(s) other than nitric oxide, are involved in the inhibitory nonadrenergic noncholinergic relaxation.

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