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Localization of leukaemia inhibitory factor to airway epithelium and its amplification of contractile responses to tachykinins

[1]Darryl Knight, Karen McKay, Barry Wiggs, R. Robert Schellenberg & Tony Bai

University of British Columbia Pulmonary Research Laboratory, St Paul's Hospital, 1081 Burrard Street, Vancouver, British Columbia V6Z 1Y6, Canada

1 In neural tissue, leukaemia inhibitory factor (LIF) is an important trophic cytokine. In this investigation, we determined if LIF was present in human and guinea-pig airways and examined the role of this cytokine in modulating airway responses to endogenous and exogenous tachykinins as well as muscarinic receptor and β-adrenoceptor stimulation.

2 The presence of LIF in both human and guinea-pig airways was determined by immunohistochemistry. Guinea-pig tracheal explants were incubated in CRML-1066 media containing LIF (0.5, 5 or 50 ng ml^{-1}) for periods of 3, 6, 24 and 48 h. Tracheal rings were then transferred to organ baths for measurement of isometric force in response to carbachol, capsaicin, the neurokinin$_1$ (NK_1) receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P (SP), the NK_2 receptor agonist neurokinin A (NKA) and isoprenaline.

3 LIF immunoreactivity was observed primarily in basally situated cells in the airway epithelium of both large and small airways. Less intense immunoreactivity was observed in vascular endothelium and glandular epithelium.

4 Treatment with LIF (0.5 ng ml^{-1}) for 3 and 6 h significantly increased contractile responses to capsaicin by 42% and 43%, respectively, compared to time controls, whereas higher concentrations of LIF (5 and 50 ng ml^{-1}) enhanced capsaicin-induced contractions only after 6 h. After 24 h, responses to capsaicin were not significantly different from 0 h control. Contractile responses to capsaicin following exposure to LIF at any concentration for 24 h were not significantly different from relative time control values.

5 Responses to [Sar^9,$Met(O_2)^{11}$]-SP, carbachol and isoprenaline were not influenced by time in culture or by exposure to LIF for up to 48 h. Contractile responses induced by NKA were not influenced by 3 or 6 h exposure to LIF, but at 24 and 48 h the mean maximum contractile responses to NKA were significantly increased by 33% and 35%, respectively, compared to control.

6 These results demonstrate that LIF is present in guinea-pig and human airway epithelium, and modulates airway responses to tachykinins. In the acute setting LIF augments the capsaicin-induced release of endogenous tachykinins, whilst in the longer term (>24 h), LIF increases airway smooth muscle responses to tachykinins via an NK_2 receptor selective mechanism. We conclude that LIF may be an important effector molecule in the response of airways to injury or inflammation.

Keywords: Cytokine; substance P; neurokinin A; capsaicin; asthma

Introduction

The tachykinin sensory neuropeptides substance P (SP) and neurokinin A (NKA) have been implicated as important mediators in many of the processes causing airway inflammation and increased airway responsiveness (Solway & Leff, 1991; Bai *et al*., 1995). SP-immunoreactive nerves have been localized within and immediately beneath the respiratory epithelium and surrounding blood vessels and mucous glands (Helke *et al*., 1990; Barnes *et al*., 1991), whilst SP (NK_1) and NKA (NK_2) receptors have been localised on numerous cell types present within the airway wall (Bai *et al*., 1995; Knight *et al*., 1996). In addition to multiple roles in the inflammatory response, these peptides may also be involved in injury repair, inducing the proliferation and migration of various cell types including epithelial cells (White *et al*., 1995), fibroblasts (Nilsson *et al*., 1985; Ziche *et al*., 1990a) and smooth muscle cells (Ziche *et al*., 1990b), all of which may contribute to more chronic responses to airway injury such as remodelling of the airway wall.

Leukaemia inhibitory factor (LIF) is a 38–67 kDa secreted glycoprotein (Gearing, 1992) which has a diverse array of biological effects ranging from the differentiation of myeloid leukaemic cells into a macrophage lineage (Tomida *et al*., 1984) to effects on bone metabolism (Ishimi *et al*., 1992), inflammation (Noda *et al*., 1990), neural development (Murphy *et al*., 1991), embryogenesis (Fry, 1992) and the maintenance of implantation (Strickland & Richards, 1992). LIF has been grouped within the interleukin-6 (IL-6) family of cytokines which also includes IL-11, ciliary neurotrophic factor, oncostatin-M and more recently, cardiotrophin-1 (Kishimoto *et al*., 1995). This classification has been based on the structural homology of this group of cytokines and common utilization of the GP130 signal transducer with their receptor complexes (Kishimoto *et al*., 1995).

Exposure of neural tissue to inflammatory stimuli (Kessler *et al*., 1993), pro-inflammatory cytokines such as IL-1β (Friedin & Kessler, 1991; Kessler *et al*., 1993) or injury (Ludlam *et al*., 1995) has been shown to increase the synthesis and release of LIF. In turn, LIF has a number of actions on these neurones including the regulation of neurotransmitter levels and their receptors. Indeed, exposure of rat cultured dorsal root ganglia to LIF has been shown to increase mRNA and protein for SP and NK_1 receptors while coordinately downregulating muscarinic receptor mRNA (Ludlam & Kessler, 1993; Ludlam *et al*., 1994).

In previous studies we have documented an upregulation of NK receptor gene expression in human airway inflammatory disorders (Bai *et al*., 1995). However, the mechanisms regulating the expression of tachykinin receptors in airways are unknown.

[1] Author for correspondence.

Based on the stimulating effects of LIF on SP and NK_1 receptor gene expression in neural tissue, we hypothesised that LIF would be present in airway tissue and could regulate the expression and activity of tachykinins and their receptors. To evaluate these hypotheses we have determined if LIF is present in the airways and with guinea-pig tracheal explants as a model, have examined the effects of LIF on responses to capsaicin, tachykinins, carbachol and isoprenaline.

Methods

Immunohistochemical localization of LIF in human bronchus and guinea-pig trachea

Tracheal rings were obtained from female guinea-pigs which were killed by an overdose of pentobarbitone according to a protocol approved by the University of British Columbia animal ethics committee. Human bronchial sections were obtained from the lung tissue bank at St. Paul's Hospital.

Formalin-fixed, paraffin embedded sections of guinea-pig trachea and human bronchus were used for the immunohistochemical detection of LIF. Slides were sequentially dewaxed and rehydrated by immersion in xylene followed by graded alcohol washes and finally immersion in distilled water. Airway sections were preincubated with normal goat serum (1:10) for 1 h to block non-specific binding of the antibody, washed in Tris buffered saline (TBS, pH 7.5) and subsequently incubated with goat anti-rabbit LIF polyclonal antibody (1:200) for 2 h at room temperature. Sections incubated with antibody that had been pre-adsorbed with an equal concentration of recombinant human LIF for 30 min served as the negative control. Following washing in TBS, sections were incubated in anti-rabbit IgG (1:20) for 30 min. Visualization of antibody binding was by the alkaline phosphatase anti-alkaline phosphatase method.

Explanted trachea

Tracheal ring explants were used as previously described (McKay *et al.*, 1995). Tracheae were removed and placed in Liebowitz L-15 medium supplemented with 100 units ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin and all connective tissue and visible blood vessels were removed. Eleven tracheal rings 1.5 – 2 mm in width were cut and rings immediately adjacent to either the larynx or the carina were discarded. One ring from each trachea was placed in organ baths for immediate use. Randomly chosen rings were allocated into two groups of 4 rings for explant culture. Within each group, one ring served as the time control, while the remaining 3 rings were incubated in the presence of 0.5, 5 or 50 ng ml^{-1} LIF for a predetermined period of time. By use of this method, the effects of LIF at two different time points could be assessed with each animal. Tracheal rings were placed individually in 35 mm plastic tissue culture dishes containing 1.5 ml of CRML-1066 medium supplemented with 2 μg insulin, 200 units penicillin, 200 μg streptomycin, 2 mM glutamine and 10% FBS, in the presence or absence of LIF at concentrations of 0.5, 5 or 50 ng ml^{-1}. Dilutions of LIF were made in a Puck's saline/0.1% bovine serum albumin solution. Tissue culture dishes were placed on a tray in a controlled atmosphere chamber which was flushed with a mixture of 45% O_2, 50% N_2 and 5% CO_2 at a flow rate of 4 l min^{-1} for 15 min. The chamber was then placed in a 37°C incubator on a rocking platform set at 10 cycles min^{-1} so that the tracheal lumen was intermittently exposed to media and gas mixture. The chamber was flushed with fresh gas mixture every 12 h.

Organ bath studies

Tracheal rings were suspended between two stainless-steel hooks in 10 ml water jacketed organ baths containing Krebs-Henseleit solution (composition (mM): NaCl 121, KCl 5.4, $MgSO_4$ 1.2, Na_2HPO_4 1.2, $NaHCO_3$ 15, $CaCl_2$ 2.5 and glucose 11.5) gassed with 95% O_2/5% CO_2. Indomethacin (5 μM) was added to the Krebs-Henseleit reservoir and was present for the duration of the experiment. Changes in isometric force in response to contractile and relaxant agonists were measured with Grass FT.O3C transducers coupled to a Beckman polygraph.

Tracheal preparations were suspended at an initial resting tension of 500 mg and allowed to equilibrate for 1.5 h. After this period, all tracheal rings were stimulated with a submaximal concentration of ACh (10 μM) at incremental resting tensions in order to determine the optimal resting tension (L_{max}). Once L_{max} was established tissues were set at this tension for the duration of the experiment. After a suitable recovery and equilibration period, all tissues were challenged with a maximal concentration of ACh (1 mM) to determine tissue responsiveness. Once the tissues had generated the maximum response, all preparations received multiple washes in fresh carbogenated, pre-warmed Krebs-Henseleit solution and were allowed to return passively to baseline tension.

Protocol 1: effect of LIF on tracheal smooth muscle responses to carbachol, capsaicin and isoprenaline

Tracheal rings were incubated with LIF (0.5, 5 or 50 ng ml^{-1}) or media alone for 0, 3, 6, 24 and 48 h. A cumulative concentration-effect curve for carbachol (0.1 μM – 1 mM) was constructed for all tissues followed by washout and passive return to baseline tension. Tissues were allowed to rest for a further 30 min in the added presence of phosphoramidon (10 μM) to inhibit the activity of endogenous neutral endopeptidases. Following this period, a cumulative concentration-effect curve was constructed for capsaicin (1 nM – 10 μM) with each addition producing log fold increases in agonist concentration. Once the maximum contraction was attained, a relaxant cumulative concentration-effect curve was constructed for the β-adrenoceptor agonist ($\pm$)-isoprenaline (1 nM – 10 μM).

Protocol 2: effect of LIF on tracheal smooth muscle responses to NK_1 and NK_2 receptor agonists

The effect of LIF on contractile responses to exogenous tachykinins were assessed by use of the selective NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-SP and the NK_2 receptor agonist NKA. Tracheal explants were incubated with LIF at the optimal concentration as determined in Protocol 1, or media alone, for 0, 3, 6, 24 or 48 h. Following transfer to organ baths, tracheal rings were incubated for a further 30 min in the presence of phosphoramidon (10 μM) before the generation of a cumulative concentration-effect curve for either [Sar^9,$Met(O_2)^{11}$]-SP (0.1 nM – 1 μM) or NKA (0.1 nM – 1 μM). Only one agonist was studied per tracheal ring and each agonist was paired against the appropriate time control.

Drugs and chemicals

The following drugs were used: polyclonal rabbit anti-human LIF antibody, recombinant human LIF (Pharmingen, San Diego, CA); [Sar^9,$Met(O_2)^{11}$]-SP, NKA (Peninsula Laboratories, Belmont, CA); capsaicin, indomethacin, ($\pm$)-isoprenaline, acetylcholine chloride, carbamylcholine chloride (carbachol), Pucks saline, phosphoramidon (Sigma chemical Co., St. Louis, MO); CRML 1066 medium and all cell culture additives were obtained from Gibco (Burlington, OR).

Analysis of results

The effects of LIF or vehicle on tracheal smooth muscle responses were normalised by expression as a percentage of the ACh (1 mM) response for each preparation. Data are presented as means $\pm$ s.e.mean. Statistical differences between control and test preparations at each time point were determined by

two-way ANOVA with Bonferroni correction performed *post-hoc* to correct for multiple comparisons. A $P<0.05$ was considered significant.

Results

Immunohistochemical localization of LIF

Immunoreactivity to LIF was observed within discrete populations of epithelial cells in both cartilaginous and membranous airways of human lung (Figure 1a and b) as well as guinea-pig tracheal sections. These cells were situated immediately adjacent to the basal lamina and were present along the entire circumference of the epithelial layer. Within these cells, LIF immunoreactivity appeared to be concentrated intracellularly. Less intense immunoreactivity was also observed in vascular endothelial cells, some sub-mucosal glands, nerve bundles and alveolar macrophages.

Effect of LIF on tracheal smooth muscle responses to carbachol

Incubation for up to 48 h in media alone did not affect the mean contractile responses of tracheal rings to a single concentration of ACh (1 mM, Table 1). Similarly, exposure to LIF (0.5–50 ng ml^{-1}) did not significantly influence tracheal responses to ACh (Table 1).

The effect of incubation time and LIF treatment on contractile responses to carbachol is shown in Table 2. Exposure to LIF at concentrations of 0.5, 5 or 50 ng ml^{-1} did not significantly alter the mean maximum contractile responses (E_{max}) to carbachol relative to the appropriate time controls. Similarly, tissue sensitivity to carbachol (pD_2) was not altered by LIF pretreatment (Table 2).

Effect of LIF on tracheal smooth muscle responses to capsaicin

Figure 2a depicts the effect of incubation time on the contractile responses to capsaicin. At time 0 h, E_{max} to capsaicin was $90\pm8\%$ of the ACh-induced contraction. Contractile responses declined with time such that after 24 h incubation in media, the maximum contraction was $27\pm5\%$ of the ACh-induced response. After 48 h in culture, contractile responses to capsaicin were absent.

Table 1 Effect of time and LIF treatment on contractile responses of guinea-pig trachea to ACh (1 mM)

Time	*Control*	*LIF-treated* 0.5 ng ml^{-1}	5 ng ml^{-1}	50 ng ml^{-1}
0 h	1513±142			
3 h	1583±232	1666±103	1050±150	1075±195
6 h	1758±82	1716±190	1083±234	1325±175
24 h	1960±293	1841±324	2141±429	2133±259
48 h	1800±380	2175±414	1525±150	1900±400

Values are expressed as means±s.e.mean mg force generated; $n=6$ animals in each group except 0 h ($n=14$). Incubation time with media with or without LIF treatment did not significantly affect contractile responses up to 48 h.

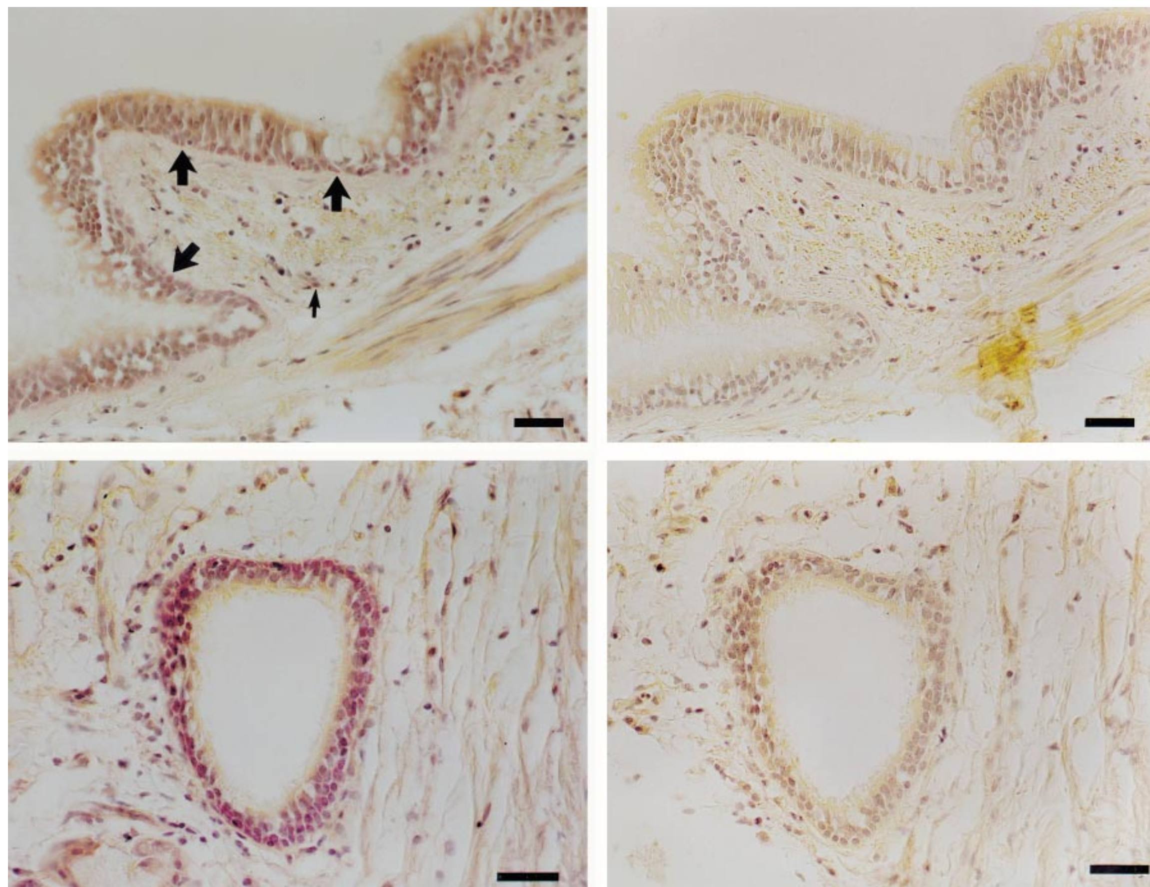

Figure 1 LIF immunoreactivity (LIF-IR) in formalin fixed, paraffin embedded sections of human airway. (a) Cartilaginous airway: LIF-IR was concentrated primarily in basal cells of the airway epithelium (🡅), with less intense immunoreactivity observed on other cell types (↑). (b) Membranous airway: LIF-IR was concentrated in basally situated epithelial cells. (c and d) Corresponding negative controls: LIF antibody was pre-adsorbed with an equal concentration of recombinant human LIF for 30 min before incubation with tissue sections. Sections are counterstained in Meyers haematoxylin. Bar = 40 μm.

Table 2 Effect of time and LIF treatment on contractile responses of guinea-pig trachea to carbachol (1 nM – 10 μM)

	Control		*LIF-treated tissues* 0.5 ng ml^{-1}		5 ng ml^{-1}		50 ng ml^{-1}	
Time	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2
0 h	126±6	7.36±0.1						
3 h	103±8	7.11±0.2	138±26	7.09±0.1	118±5	7.07±0.2	119±17	6.98±0.2
6 h	116±14	6.91±0.1	123±11	7.00±0.1	126±11	6.85±0.1	108±4	7.00±0.1
24 h	104±18	6.98±0.2	124±6	7.31±0.2	107±10	7.28±0.2	121±7	6.98±0.2
48 h	124±130	6.86±0.3	111±7	6.86±0.3	109±18	6.97±0.3	112±7	6.84±0.2

Values are expressed as means±s.e.mean; $n=6$ animals in each group except for 0 h ($n=14$). E_{max} = maximum contractile response to carbachol and is expressed as a percentage of responses to ACh (1 mM). $pD_2 = -\log EC_{50}$. Incubation time with media with or without LIF treatment did not significantly alter the contractile responses to carbachol.

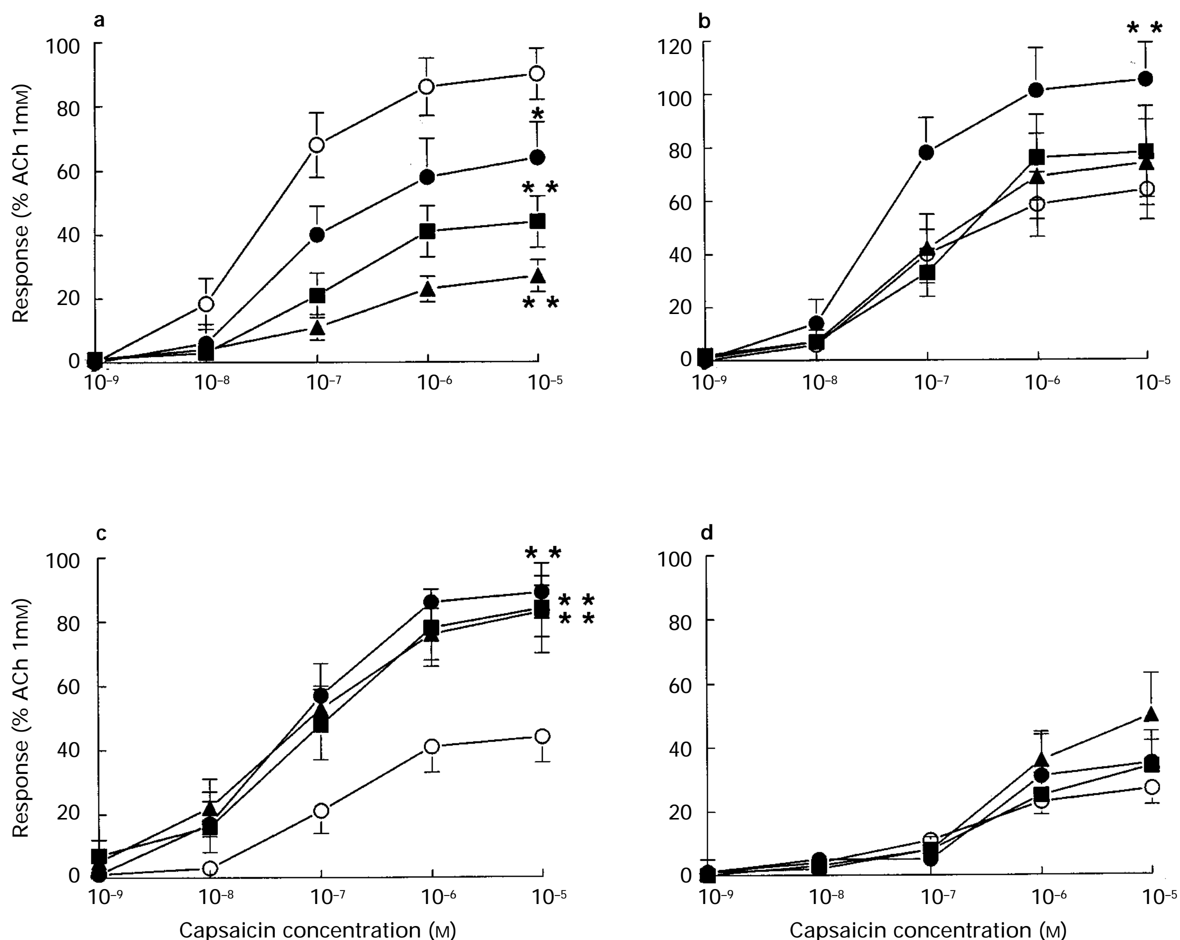

Figure 2 Effect of incubation time in media alone or media and LIF on contractile respones of guinea-pig tracheal explants to capsaicin. (a) Incubation time in media alone was for 0 h (○), 3 h (●), 6 h (■) and 24 h (▲). *$P<0.05$, **$P<0.001$ compared to 0 h group. (b) Effect of 3 h incubation with media and LIF 0.5 ng ml^{-1} (●), 5 ng ml^{-1} (■) and 50 ng ml^{-1} (▲). **$P<0.001$ compared to 3 h control group (○). (c) Effect of 6 h incubation with LIF 0.5 ng ml^{-1} (●), 5 ng ml^{-1} (■) and 50 ng ml^{-1} (▲). **$P<0.001$ compared to 6 h control group (○). (d) Effect of 24 h incubation with LIF 0.5 ng ml^{-1} (●), 5 ng ml^{-1} (■) and 50 ng ml^{-1} (▲) compared to 24 h control group (○). Experiments were performed in the presence of indomethacin (5 μM) and phosphoramidon (10 μM). Data are shown as mean for $n=6$ animals, except at 0 h where $n=14$; vertical lines show s.e.mean.

The effects of incubation with LIF on contractile responses to capsaicin are shown in Figure 2b–d. Following 3 h incubation with LIF (0.5 ng ml^{-1}) (Figure 2b), E_{max} to capsaicin was 105±15% of the ACh response which was significantly greater than the appropriate time control, being 63±14% ($P<0.05$). However, after 3 h, the higher concentrations of LIF (5, 50 ng ml^{-1}) did not significantly alter the contractile responses to capsaicin (Figure 2b).

Figure 2c shows the effect of 6 h incubation with LIF on tracheal responses to capsaicin. E_{max} responses to capsaicin were 89±5%, 84±14% and 83±8% of ACh-induced contraction for preparations exposed to LIF 0.5, 5 and 50 ng ml^{-1}, respectively. These values were all significantly greater ($P<0.001$) than contractile responses to capsaicin observed in the 6 h control group. Incubation for 24 h with LIF (0.5–50 ng ml^{-1}), did not influence the E_{max} to capsaicin relative to the 24 h control response (Figure 2d).

Effect of LIF on tracheal smooth muscle responses to isoprenaline

At time 0, the β-adrenoceptor agonist isoprenaline relaxed tracheal rings to a maximum of 129±10% of the induced contraction and the magnitude of relaxation was not influ-

Table 3 Effect of time and LIF treatment on relaxant responses of guinea-pig trachea to isoprenaline

	Control		LIF-treated tissues 0.5 ng ml^{-1}		5 ng ml^{-1}		50 ng ml^{-1}	
Time	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2
0 h	129±10	7.84±0.2						
3 h	166±13	7.71±0.3	118±12	7.52±0.1	171±30	7.67±0.3	136±8	8.36±0.4
6 h	121±8	7.30±0.3	103±5	7.30±0.3	115±9	7.36±0.3	111±23	7.04±0.2
24	105±23	7.50±0.4	91±11	6.98±0.3	150±41	7.79±0.5	95±8	7.44±0.3

Values are expressed as means±s.e.mean; $n=5$ for each group except 0 h ($n=13$). $E_{max}=$ maximum relaxant effect and is expressed as a percentage of the induced contraction. $pD_2=-\log EC_{50}$. Incubation time alone or with LIF did not significantly alter relaxant responses of guinea-pig trachea to isoprenaline.

Table 4 Effect of time and LIF (0.5 ng ml^{-1}) treatment on tissue sensitivity (pD_2) to neurokinin A (NKA) and [Sar^9, $Met(O_2)^{11}$]-substance P

	[Sar^9,$Met(O_2)^{11}$]-substance P		NKA	
Time	*Control*	*LIF-treated*	*Control*	*LIF-treated*
0 h	8.00±0.1		8.71±0.1*	
3 h	7.81±0.1	7.78±0.1	8.45±0.1	8.38±0.1
6 h	7.80±0.2	7.78±0.1	8.49±0.1	8.41±0.1
24 h	7.94±0.1	8.08±0.1	8.55±0.1	8.53±0.1
48 h	8.15±0.1	8.11±0.2	8.41±0.1#	8.42±0.1

Values are expressed as means±s.e.mean; $n=6$ animals for each group except 0 h ($n=13$). pD_2 values are derived from $-\log_{10}(EC_{50})$. pD_2 value for NKA significantly different from corresponding value for [Sar^9,$Met(O_2)^{11}$]-substance P ($P<0.05$). #pD_2 value for NKA after 48 h in culture was significantly lower compared to value for 0 h control ($P<0.05$).

enced by incubation time alone (Table 3). Similarly, tissue sensitivity to isoprenaline was not significantly altered by up to 24 h in culture. Exposure to LIF (0.5, 5, 50 ng ml^{-1}) for up to 24 h did not influence either the maximum relaxant response or the tissue sensitivity to isoprenaline (Table 3). Since tracheal explants did not respond to capsaicin after 48 h in culture, a small number of tracheal rings ($n=3$) were precontracted with carbachol. Relaxant responses to isoprenaline were not different between tracheal preparations exposed to LIF (0.5, 5, 50 ng ml^{-1}) and the appropriate time control (data not shown).

Effect of LIF on tracheal smooth muscle responses to [Sar^9,$Met(O_2)^{11}$]-SP

Contractile responses to the selective NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-SP were not influenced by time in culture (Table 4). Thus, at time 0 h control responses were 88±7% of the ACh-induced contraction and after 48 h in culture maximum contractile responses were 87±8% (Table 4). The effect of LIF (0.5 ng ml^{-1}) on contractile responses to [Sar^9,$Met(O_2)^{11}$]-SP are shown in Figure 3a–d. Exposure of tracheal rings to LIF for up to 48 h did not influence the maximum contraction elicited by [Sar^9,$Met(O_2)^{11}$]-SP (Table 4). Similarly, tissue sensitivity to [Sar^9,$Met(O_2)^{11}$]-SP was not significantly influenced by exposure to LIF relative to the appropriate time control (Figure 3a–d).

Effect of LIF on tracheal smooth muscle responses to NKA

At time 0 h, the maximum contractile response to the NK_2 receptor agonist NKA was 107±7% of the ACh-induced response and after 48 h was 92±7%, which was not statistically significant ($P=0.3$). Incubation of tracheal rings for 48 h in culture media alone reduced tissue sensitivity to NKA, as reflected by a lower pD_2 value ($P<0.05$; Table 4).

Exposure of tracheal preparations to LIF (0.5 ng ml^{-1}) for up to 6 h did not affect contractile responses to NKA in terms of either E_{max} or tissue sensitivity (Figure 4a,b). However, after 24 h exposure to this concentration of LIF, the maximum contractile response to NKA was significantly increased by 32% compared to the parallel time control ($P<0.001$; Figure 4c, Table 4). This level of enhancement was maintained in tracheal rings exposed to LIF for 48 h ($P<0.001$; Figure 3d). However at both time periods, LIF did not affect tissue sensitivity to NKA (Figure 4c,d; Table 4).

Discussion

The results of the present investigation demonstrate that LIF is present in airway tissue, predominantly in basal cells of airway epithelium and augments the contractile responses to tachykinins released endogenously by capsaicin as well as to exogenous tachykinins acting through NK_2 receptors. The differing time course of the enhanced effects to capsaicin and NKA suggest multiple mechanisms of action of LIF, with the former representing an enhanced release of tachykinins and the latter a time-dependent enhancement of NK_2 receptor expression. The effects of LIF appear selective for tachykinins, since contractile responses to the muscarinic receptor agonist, carbachol, and relaxant responses to the β-adrenoceptor agonist, isoprenaline, were not affected by treatment with this cytokine. Upregulation of tachykinin-induced responses induced by LIF is consistent with its effects observed in neural ganglia following injury or exposure to pro-inflammatory cytokines (Ludlam *et al*., 1994) and suggest that LIF may be an important cytokine in the response of airways to injury or inflammation.

In keeping with its biological activities in implantation, pregnancy (Fry, 1992; Arici *et al*., 1995) and neural development (Murphy *et al*., 1991), LIF gene expression and protein has been localized to the uterine glandular epithelium (Arici *et al*., 1995; Chen *et al*., 1995), breast epithelium (Estrov *et al*., 1995), neural tissue (Kessler *et al*., 1993) and endothelial cells (Lubbert *et al*., 1992). However, within pulmonary tissue, LIF expression has only been examined in cultured lung fibroblasts (Elias *et al*., 1994). The results of this study provide evidence that LIF is also present within airway epithelial cells of human bronchus and guinea-pig trachea. The profile of immunoreactivity in these sections localised LIF to distinct populations of basally situated cells. The function of these cells is not known, although it is possible that they may act in a similar fashion to undifferentiated cells of the epidermis and function as primary stem cells from which mucous secreting and ciliated cells are derived (Ayers & Jeffrey, 1988). Indeed, there is increasing evidence to support the hypothesis that LIF and related cytokines play both direct and indirect roles in the proliferative response of both epithelial and mesenchymal cells. For example, IL-6 has been shown to stimulate hyperplasia of cultured airway smooth muscle cells directly (De *et al*., 1995) and is essential for platelet-derived growth factor (PDGF)-induced proliferation of primary, non-transformed cultures of human vascular smooth muscle cells and lung fibroblasts taken from biopsy (Roth *et al*., 1995). In uterine

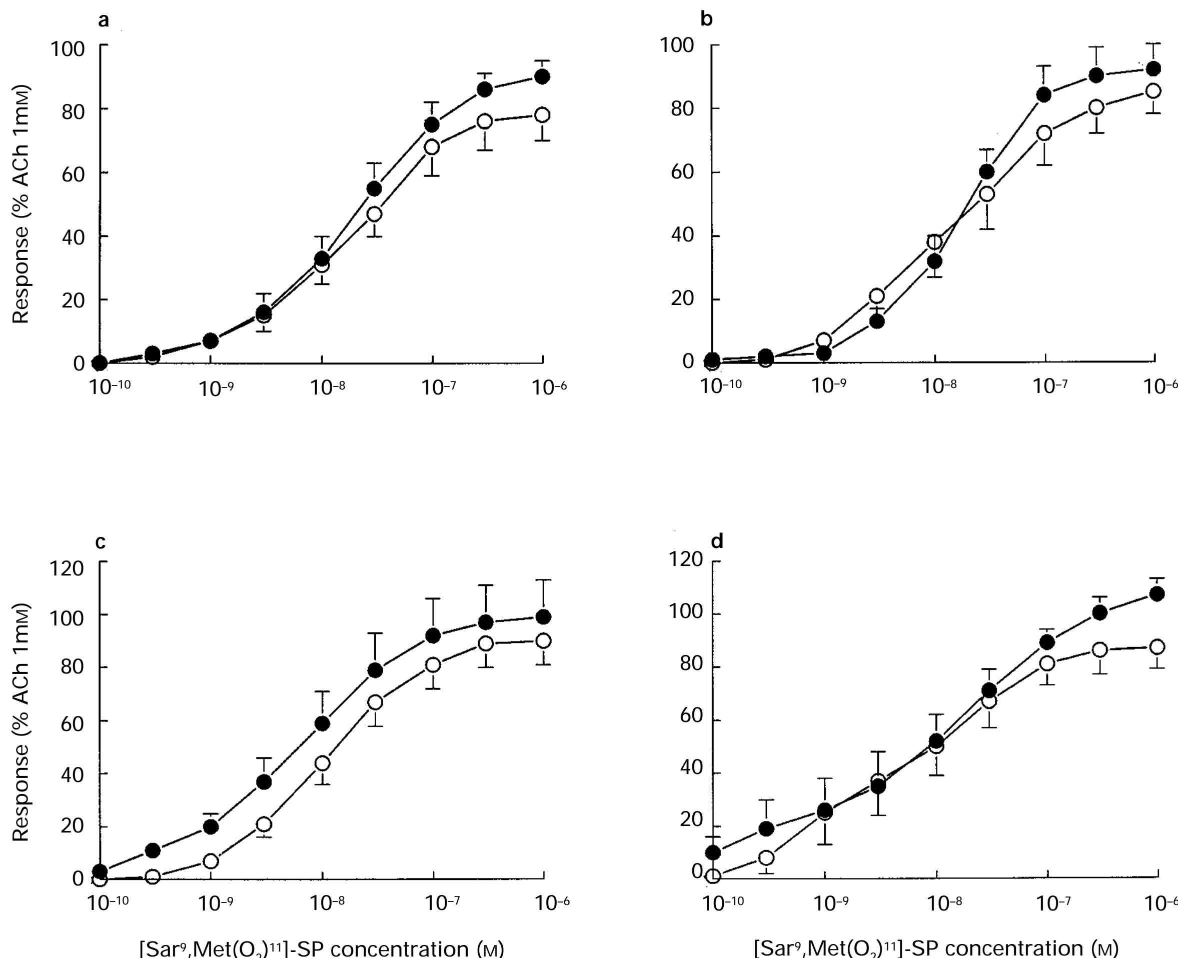

Figure 3 Effect of incubation time in media alone and in the added presence of LIF on contractile responses of guinea-pig tracheal explants to [Sar^9,Met(O$_2$)^{11}]-SP. (a) Effect of incubation for 3 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). (b) Effect of incubation for 6 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). (c) Effect of incubation for 24 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). (d) Effect of incubation for 48 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). Experiments were performed in the presence of indomethacin (5 μM) and phosphoramidon (10 μM). Data are shown as mean for $n=6$ animals; vertical lines show s.e.mean.

endometrium, LIF has been shown to induce the secretion of fibronectin by trophoblasts, which in turn aids in implantation (Strickland & Richards, 1992). In airway tissue, fibronectin is produced by epithelial and mesenchymal cells and is an important signal protein involved in the proliferation, migration and attachment of cells at sites of injury and inflammation (Rickard *et al.*, 1993). LIF is also produced by mast cells isolated from the rat (Marshall *et al.*, 1993). Given the proximity of mast cells to sensory nerves (Marshall & Waserman, 1995) and the epithelium (Lamb & Lumsden, 1982), the ability of mast cells to produce LIF may also be an important factor in the proliferative response following mucosal damage.

Incubation of dorsal root ganglia with LIF has been shown to result in a marked increase in the expression of both SP mRNA and NK$_1$ receptor mRNA (Kessler *et al.*, 1993; Ludlam & Kessler, 1993). Thus, we hypothesised that some of the effects of LIF would involve the transcription of new receptors and would thus require prolonged periods in culture. In the current investigation, we have used a tracheal explant model in which morphological and physiological integrity can be maintained for at least 7 days (McKay *et al.*, 1995). Incubation of guinea-pig tracheal rings with LIF for periods of 3 and 6 h produced a profound increase in the contractile response to capsaicin, compared to the relevant time control. The relatively short time period in which these augmented responses were observed suggest that LIF may increase the capsaicin-induced release of tachykinins from nerve terminals. After 24 h in culture, contractile responses to capsaicin were much lower than the initial responses at 0 h and LIF failed to influence this diminished response, suggesting neuronal degeneration. Indeed, SP and related peptides are synthesized in cell bodies within the jugular ganglia or spinal cord and are transported axonally to peripheral nerve terminals (Helke *et al.*, 1990; Solway & Leff, 1991; Kummer *et al.*, 1992). No cell bodies for sensory neurones have been described in lung tissue. The current study further supports these conclusions in that after 48 h in culture, responses to capsaicin were non-existent, whereas responses to muscarinic receptor activation were unchanged.

In contrast to the effects of capsaicin, contractile responses to the NK$_2$ receptor agonist NKA were not affected by exposure to LIF for up to 6 h. However, after 24 and 48 h exposure to LIF, the contractile responses to NKA were significantly augmented. The delayed increase observed for NKA mediated contractions may be due to an increased synthesis of tachykinin receptors. In support of this, Lewis (1994) demonstrated that in neural tissue, exposure to LIF for periods in excess of 6 h are required for a significant increase in tachykinin receptor mRNA. However, receptor binding studies are required for final confirmation of this hypothesis. The lack of effect of [Sar^9,Met(O$_2$)^{11}]-SP suggests an effect of LIF only on NK$_2$ receptors resident on airway smooth muscle. However, these findings do not preclude an effect of LIF on NK$_1$ receptors on other cell types. Since the expression of both NK$_1$ and NK$_2$ receptors is upregulated

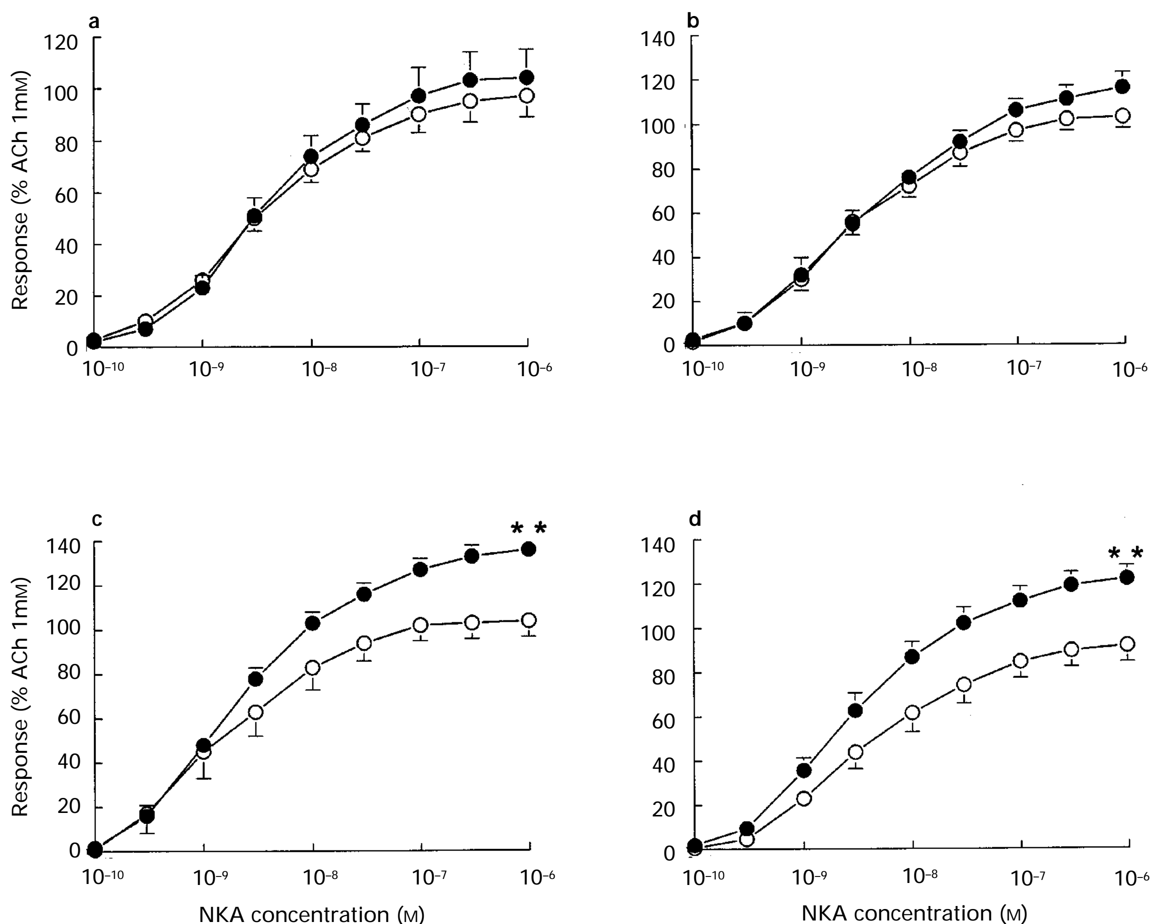

Figure 4 Effect of incubation time in media alone and in the added presence of LIF on contractile responses of guinea-pig tracheal explants to NKA. (a) Effect of incubation for 3 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). (b) Effect of incubation for 6 h with media (○) or media with LIF 0.5 ng ml^{-1} (●). (c) Effect of incubation for 24 h with media (○) or media and LIF, 0.5 ng ml^{-1}. $^{**}P<0.001$, compared to 24 h control group. (d) Effect of incubation for 48 h with media (○) or media and LIF 0.5 ng ml^{-1} (●). $^{**}P<0.001$, compared to 48 h control group. Data are shown as mean and vertical lines indicate s.e.mean ($n=6$ animals). Experiments were performed in the presence of indomethacin (5 μM) and phosphoramidon (10 μM).

by airway inflammation (Bai *et al.*, 1995), further investigations into the expression and activity of NK_1 receptors on other cell types in the airways and the role of LIF are required.

Incubation of sympathetic cervical ganglia with LIF has been shown to down regulate mRNA for muscarinic receptors coordinate with an increase in both SP and NK_1 receptor mRNA (Ludlam & Kessler, 1993; Ludlam *et al.*, 1994). Thus, it was of interest to determine the effects of LIF on muscarinic responses in airway tissues. Contractile responses to carbachol in both control and LIF treated tracheal rings were not altered by up to 48 h in culture, suggesting that LIF does not influence muscarinic receptor expression and function on airway smooth muscle. Exposure of tracheal rings to LIF for periods of up to 24 h did not significantly influence the relaxant responses to isoprenaline, when tachykinins were used to contract the airway smooth muscle. The effects of other pro-inflammatory cytokines on airway responses to β-adrenoceptor agonists are controversial. For example, IL-1β and tumour necrosis factor α (TNFα) have been shown to either reduce the maximum relaxant effects of isoprenaline (Wills-Karp *et al.*, 1993) or have no effect (Van Oosterhout *et al.*, 1992).

Capsaicin selectively stimulates sensory afferent fibres to release neuropeptides such as SP and NKA, and in turn these mediators contract airway smooth muscle via an interaction with specific receptors (Solway & Leff, 1991). The relative contribution of these receptors in causing airway smooth muscle contraction is species dependent. In human airways, contractile responses to capsaicin and SP and NKA are thought to be mediated through the NK_2 receptor (Naline *et al.*, 1989; Ellis *et al.*, 1993). In guinea-pig trachea, stimulation of both NK_2 and NK_1 receptors are thought to mediate the bronchoconstrictor response to capsaicin (Bertrand *et al.*, 1993; Lilly *et al.*, 1994) and the related compound resiniferatoxin (Foulon *et al.*, 1993), although NK_2 receptor mediated pathways appear to predominate (Mizuguchi *et al.*, 1996). In the present study, contractile responses to NKA were significantly greater than those elicited by the selective NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]-SP. The observation that exposure to LIF augmented contractile responses to NKA but did not alter contractile responses to [Sar^9,$Met(O_2)^{11}$]-SP at any time point, suggest that on guinea-pig tracheal smooth muscle, LIF may selectively act on the NK_2 receptor.

In summary, this study has demonstrated the presence of LIF within epithelial cells of human and guinea-pig airways. Incubation of tracheal explants with LIF did not influence carbachol-induced contractions or β-adrenoceptor-mediated relaxation. However, LIF significantly increased the contractile response to capsaicin, suggesting that in acute settings LIF facilitates the release of tachykinins from airway sensory nerves, while in the longer term LIF may produce direct effects on the expression of tachykinin receptors in the airways. The location of LIF coupled with its ability to upregulate significantly the release of, and response to, tachykinins suggests it may be an important effector molecule in the airway response to injury or inflammation.

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