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THE EFFECT OF LEUKOTRIENE C_4 ON MUCIN RELEASE INTO THE CAT TRACHEA *in vivo* AND *in vitro*

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We have tested the effect of leukotriene C_4 (LTC_4, 6×10^{-8} to 6×10^{-5} M) on the output of radiolabelled mucins from the trachea of the anaesthetized cat. Doses between 6×10^{-7} M and 6×10^{-5} M stimulated mucin release. FPL 55712 (9.5×10^{-6} M) partially antagonized the effect of the highest dose of LTC_4. Tests of LTC_4 (6×10^{-8} to 6×10^{-6} M) on cat trachea *in vitro* failed to show any effect on mucin secretion. We conclude that leukotrienes may be one of the mediators of mucus secretion into the inflamed airway.

Introduction The inflamed airway secretes mucus at an increased rate (Richardson & Phipps, 1978) and a number of mediators of inflammation are known to augment mucus secretion. These include histamine (Phipps, Richardson, Corfield, Gallagher, Jeffery, Kent & Passatore, 1977; Shelhamer, Marom & Kaliner, 1980) and certain prostaglandins (Richardson, Phipps, Balfre and Hall, 1978; Marom, Shelhamer & Kaliner, 1981). There is indirect evidence that lipoxygenase products which include leukotrienes may stimulate mucin secretion (Marom *et al.*, 1981). Leukotriene (LT) D_4 has been detected in sputum from patients with cystic fibrosis (Cromwell, Walport, Morris, Taylor, Hodson, Batten & Kay, 1982). In this paper we describe experiments to test whether LTC_4 stimulates mucin secretion into the cat trachea.

Methods In this study both *in vivo* and *in vitro* experiments were performed.

In vivo *experiments* Mucins radiolabelled with ^{35}S and ^{3}H were collected at 15 min intervals from a segment of cervical trachea in the pentobarbitone-anaesthetized cat (Peatfield, Hall, Richardson & Jeffery, 1982). After exhaustive dialysis to remove unbound radiolabel all samples were dialysed against 6M urea to bring insoluble mucus into solution (Peatfield & Richardson, 1982). LTC_4 (6×10^{-8} to 6×10^{-5} M) was mixed with the Krebs-Henseleit wash fluid and given at hourly intervals for one collection period. In the intervening collection periods the tracheal segment remained filled with Krebs-Henseleit solution. In 4 experiments FPL 55712 (sodium 7-[3(4-acetyl-3-hydroxy-2 propylphenoxy)-2-hydroxypropoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate, 1.9×10^{-6} M, 1 cat; 9.5×10^{-6} M, 3 cats), an antagonist of slow reacting substance of anaphylaxis (SRS-A)/leukotrienes, was added to the wash fluid and administered throughout the experiment. In 3 cats we recorded blood pressure, tidal volume and respiratory frequency.

In vitro *experiments* Five pieces of trachea from 3 cats were mounted in Ussing chambers, (Phipps, Nadel & Davis, 1980), 2.0 mCi [^{35}S]-sulphate was added to the submucosal chamber and radiolabelled mucins collected by draining the luminal chamber every 15 min. In these experiments LTC_4 was added to both chambers for one 15 min period at hourly intervals in ascending concentrations from 6×10^{-8} M to 6×10^{-6} M. At the end of each experiment an agonist known to stimulate mucin production (phenylephrine 8×10^{-5} M, $n = 2$; methacholine 5×10^{-5} M, $n = 3$) was given.

Calculation of results After dialysis, aliquots of tracheal washings were mixed with scintillation fluid and counted in triplicate by Intertechnique SL-30 counter. The ^{35}S and ^{3}H content of samples were calculated and expressed as (disintegrations per s) and per min ($d\,s^{-1}\,min^{-1}$) of secretion (Peatfield *et al.*, 1982). Percentage changes in output of radioactivity for LTC_4-stimulated samples over bracketing control samples were calculated.

Results Figure 1 shows that the higher doses of LTC_4 increased the rate of secretion of mucins labelled with both radioisotopes. For ^{3}H-radiolabelled mucins the effects were significant at concentrations of 6×10^{-7} M and higher, for ^{35}S-radiolabelled mucins at 6×10^{-6} M and above. Washings from tracheae treated with higher doses of LTC_4 consistently contained more visible mucus than did adjacent control samples. The effects appeared to be dose related (Figure 1). Administration of LTC_4 caused no changes in blood pressure, tidal volume or respiratory frequency.

In the presence of FPL 55712, LTC_4 still increased the output of mucins. Only at the highest dose of LTC_4 was there any diminution of effect in cats treated with the antagonist (Figure 1). The diminu-

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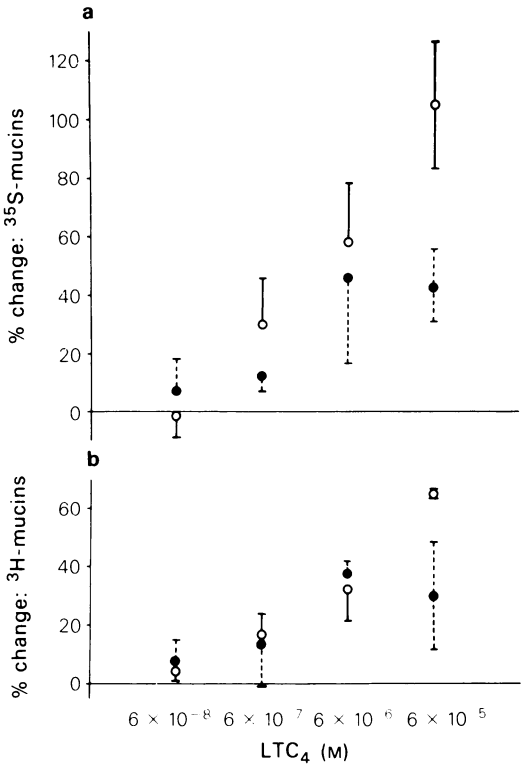

Figure 1 Stimulation of radiolabelled mucin output by leukotriene C_4 (LTC_4) and the effect of FPL 55712 on the response. Ordinates: (a) percentage change in output of ^{35}S-labelled mucins; (b) percentage change in output of ^{3}H-labelled mucins. Abscissae: molar concentrations of LTC_4, log scale. (○) LTC_4 without antagonist ($n = 5$); (●) LTC_4 in the presence of FPL 55712, 9.5×10^{-6} M ($n = 3$). Bars show 1 standard error.

tion was significant for ^{3}H-labelled mucins ($P < 0.05$), but not for ^{35}S-mucins ($0.10 > P > 0.05$).

In 5 tissues we tested the effects of LTC_4 *in vitro*. Even at the highest dose (6×10^{-6} M) the agonist had no effect (mean increase in ^{35}S-labelled mucins = $10.2 \pm 8.7\%$). In each experiment the reference drugs stimulated mucin output (range +82% to +205%).

Discussion These results show that LTC_4 caused a dose-related increase in airway mucin production in the cat *in vivo*, but that *in vitro* such an effect was apparently absent. This suggests that the agonist may have had an indirect effect, e.g. by changing the local circulation or by eliciting reflexes (Phipps & Richardson, 1976). However, absence of changes in pattern of breathing or blood pressure gave no evidence for the latter mechanism.

In these experiments the response of ^{35}S-labelled mucins was usually greater than ^{3}H-labelled mucins. This suggests that the submucosal glands, which take up ^{35}S avidly, were the main site of action of LTC_4 (Jeffery, 1978).

In these experiments FPL 55712 was given in high doses and this drug, which possesses only some of the characteristics of a competitive antagonist (Augstein, Farmer, Lee, Sheard & Tattersall, 1973), significantly lessened the effect of only the highest dose of LTC_4.

Recently Ahmed, Greenblatt, Birch, Marchete & Wanner (1981) reported that antigen challenge to the lungs of allergic patients slows mucociliary transport in the lungs but that treatment with FPL 55712 protects against this slowing. It is possible that the mucus released in response to leukotrienes has physical properties which make it poorly transported (King, 1979).

At the highest dose used here LTC_4 approximately doubled the secretory rate of ^{35}S labelled mucins. This effect is small compared to those of prostaglandin E_1 (PGE_1) and $PGF_{2\alpha}$ (Richardson *et al.*, 1978). Leukotrienes may, however, interact with other agonists as occurs in the skin where vasodilator prostaglandins potentiate exudation of plasma caused by LTD_4 (Peck, Williams & Piper, 1981). The increased secretion elicited by leukotrienes may be important in a number of lung diseases. This is particularly likely in asthma where there is slowed mucociliary transport and there is evidence that plugs of mucus obstruct the airway (Dunnil, 1960).

Note Since the preparation of this manuscript Shelhamer, J.H., Marom, Z., San, F., Bach, M. & Kaliner, M. (1982), *Chest*, **81**, 36S–37S, have reported that LTC_4 and LTD_4 stimulate mucin secretion from human bronchus *in vitro* and that FPL 55712 antagonizes this effect.

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