Br. J. Pharmac. (1982) **77**, 570–572

MODIFICATION OF HUMAN AIRWAY SMOOTH MUSCLE REACTIVITY BY DRUGS THAT INTERFERE WITH ARACHIDONIC ACID METABOLISM

JOHN J. ADCOCK & LAWRENCE G. GARLAND

Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Histamine-induced contractions of small airways from human lung were substantially augmented by the cyclo-oxygenase inhibitor, indomethacin, whereas the contraction of larger airways was not. Mixed cyclo-oxygenase/lipoxygenase inhibitors of arachidonic acid metabolism did not modify histamine-induced contractions of smooth muscle from either small or large airways but completely reversed the augmentation of histamine responses produced by indomethacin on the smaller airways. The SRS-A (slow reacting substance of anaphylaxis) antagonist, FPL 55712, did not affect the augmentation of histamine-induced contractions by indomethacin.

Introduction There have been several reports that indomethacin augments histamine-induced contractions of guinea-pig tracheal smooth muscle (Orehek, Douglas & Bouhuys, 1975; Adcock & Garland, 1980; Burka & Paterson, 1980). This augmentation was reversed by two compounds, CLI and BW 755c, that inhibit both the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism (Adcock & Garland, 1980). These latter observations led Adcock & Garland (1980) to suggest that the increased reactivity to histamine produced by indomethacin was due to increased synthesis of a lipoxygenase product(s) when the cyclo-oxygenase pathway was blocked. Since this phenomenon might be analogous to either aspirin sensitivity or to non-specific airway hyper-reactivity in asthmatics, we have investigated whether histamine-induced contractions of human bronchial spirals are similarly modified by inhibitors of arachidonic acid metabolism.

Methods Human bronchial tissue was obtained from patients undergoing surgery for bronchial carcinoma. Bronchioles were dissected from apparently normal lung tissue and placed in Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5. The tissues were classified into two groups according to size i.e. <3 mm and 5–8 mm internal diameter. Each bronchiole was cut to form a spiral, suspended in an organ bath and superfused at a rate of 3 ml/min with Tyrode solution at 37°C, gassed with 95% O_2 and 5% CO_2. It was then equilibrated under an initial tension of 4–5 g for 1.5 h. The load was reduced to 3 g just before the start of the experiment and responses were measured isotonically using a Harvard heart/smooth muscle transducer linked to a Rikadenki 4-channel desk-top pen recorder. At least two cumulative dose-response curves for histamine were obtained before the superfusion of test compound(s) which was usually for 20 min before and during the next histamine dose-response curve. Responses are expressed as percentages of the maximal control response.

Compounds studied were: indomethacin; BW 755c (3-amino-l-[m(trifluoromethyl)-phenyl]-2-pyrazoline, Higgs, Copp, Denyer, Flower, Tateson, Vane & Walker, 1978); CLI (3-amino-l-[p-(chlorophenyl)]-2-pyrazoline, Adcock, Garland, Moncada & Salmon, 1978) and FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4-H-l-benzopyran-2-carboxylate, Augstein, Farmer, Lee, Sheard & Tattersall, 1973).

Results The responsiveness of small airways (internal diameter <3 mm) was compared with that of larger airways (internal diameter 5–8 mm). There was no apparent difference in the sensitivity of the different sized airways to histamine. The dose-response curve occurred within the range 0.1 to 1,000 μg ($ED_{50} = 66 \pm 10$ μg) which was very similar to that reported previously for guinea-pig trachea (Adcock & Garland, 1980). In larger airways, obtained from four separate specimens of lung, indomethacin (1 μg/ml) had no effect on responses to histamine. However, in the smaller airways taken from these same lungs as well as from three others (7 specimens total), indomethacin (1 μg/ml) substantially augmented histamine-induced contractions (Figure 1a). The maximum response after indomethacin was between 3 and 4 times greater than control, an effect that was some 2–3 times greater than that found previously in guinea-pig trachea (Adcock & Garland, 1980). In human as well as in guinea-pig tissue the effect of indomethacin persisted for up to 3 h after its removal from the system.

In contrast to indomethacin, compounds CLI and BW 755c did not modify histamine-induced contractions of spirals taken from smaller airways. When

0007-1188/82/120570-03 $01.00

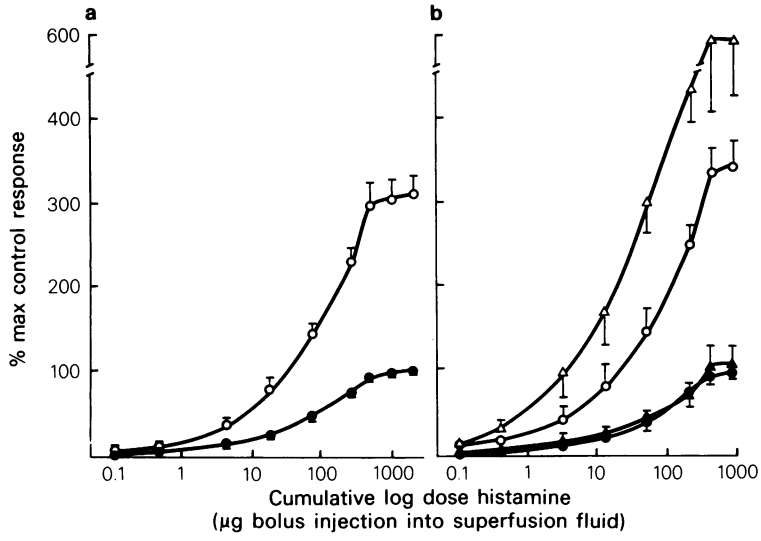

Figure 1 Histamine-induced contractions of smooth muscle spirals, taken from the small airways of man, expressed as cumulative log dose-response curves either before (●, $n = 7$ in (a), 3 in (b)) or after (○, $n = 7$ in (a), 3 in (b)) treatment with indomethacin (1 μg/ml). In (b) histamine responses after 20 min superfusion of indomethacin concomitantly with BW 755c (40 μg/ml) on the same preparation are shown by (▲) ($n = 3$). Following removal of BW 755c, indomethacin (1.0 μg/ml) superfusion was continued for a further 20 min (△; $n = 3$). Responses are expressed as percentages of the maximal control response and vertical lines show s.e.mean.

either of these phenyl pyrazolines, at concentrations between 20 to 40 μg/ml, was superfused over a bronchial spiral for 20 min before and during a histamine dose-response curve, the contractions were virtually unchanged from the preceding control responses. However, when superfused concomitantly with indomethacin (1 μg/ml), BW 755c (40 μg/ml) completely reversed the previously augmented histamine responses, obtained with indomethacin alone. (Figure 1b). This effect of BW 755c was reversible. Thus when superfusion of BW 755c was stopped but indomethacin treatment continued for a further 20 min, the histamine responses were again augmented (Figure 1b). Compound CLI was similarly effective in 3 experiments. These results with compound BW 755c and CLI in human peripheral airways are similar to those obtained previously in guinea-pig trachea (Adcock & Garland, 1980).

It has been shown that leukotrienes C_4 and D_4, products of the lipoxygenase pathway, contract human airway smooth muscle at concentrations of 10^{-10} to 10^{-5} M (Dahlen, Hedqvist, Hammarstrom & Samuelsson, 1980). This effect is blocked by the antagonist of slow reacting substance of anaphylaxis (SRS-A), FPL 55712 (Dahlen *et al.*, 1980). We therefore examined the effect of FPL 55712 on indomethacin-induced hyper-reactivity in the small airways from human lung. Contractions induced by partially-purified SRS-A (guinea-pig lung) were blocked by FPL 55712 at a concentration (0.5 μg/ml) that left histamine responses unchanged. Furthermore, the same concentration of FPL 55712 had no effect on histamine contractions previously augmented by indomethacin (1 μg/ml). In addition, when bronchial spirals were pretreated for 20 min with FPL 55712 (0.5 μg/ml) before indomethacin treatment, the characteristic augmentation was still obtained with indomethacin.

Discussion The present results demonstrate that histamine-induced responses of the small airways (less than 3 mm internal diameter) in human lung are augmented by indomethacin to an even greater extent than histamine responses of guinea-pig trachea (Orehek *et al.*, 1975; Adcock & Garland, 1980; Burka & Paterson, 1980). The lack of effect of indomethacin on histamine contractions of the larger airways in human lung (5–8 mm internal diameter) is in agreement with the observations of Brink, Grimaud, Guillot & Orehek (1980) who showed that indomethacin did not modify histamine contractions of human bronchial spirals taken from airways of 4–6 mm internal diameter. It seems, therefore, that human airways exhibit an anatomical difference in their responsiveness to indomethacin. This might be related to the functional differences between the smaller and larger airways since constriction of the smaller airways mainly affects pulmonary com-

pliance whereas constriction of the larger airways is associated predominantly with pulmonary resistance. It is of interest to note that lipoxygenase products, particularly leukotriene C_4, markedly reduced pulmonary compliance but had little effect on pulmonary resistance in the monkey. (Smedegard, Hedqvist, Dahlen, Revenas, Hammarstrom & Samuelsson, 1982).

The results obtained with two compounds that are dual inhibitors of the cyclo-oxygenase and the lipoxygenase pathways of arachidonic acid metabolism suggest that the increased responsiveness of lower airways in human lung might be attributed to an effect of lipoxygenase products. It can be envisaged that synthesis of these increases after cyclo-oxygenase inhibition, following diversion of arachidonate metabolism to the lipoxygenase pathway. However, the lack of effect of FPL 55712 on the hyper-reactivity described both in the present experiments and previously on the guinea-pig trachea (Adams & Lichtenstein, 1979; Adcock & Garland, 1980; Hitchcock, 1980) indicates that leukotrienes C_4 or D_4 are not involved in the indomethacin-induced augmentation of histamine contractions of airway smooth muscle.

The present results in human tissue support the hypothesis that lipoxygenase products (but not leukotrienes C_4 or D_4) may have a role either in non-specific airway hyper-reactivity, or in the syndrome referred to as 'aspirin-sensitive asthma'.

References

ADAMS, G.K. & LICHTENSTEIN, L. (1979). *In vitro* studies of antigen induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitised guinea-pig and human airways to antigen. *J. Immunol.*, **122**, 555–562.

ADCOCK, J.J. & GARLAND, L.G. (1980). A possible role for lipoxygenase products as regulators of airway smooth muscle reactivity. *Br. J. Pharmac.*, **69**, 167–169.

ADCOCK, J.J., GARLAND, L.G., MONCADA, S. & SALMON, J.A. (1978). Mechanism of enhancement by fatty acid hydroperoxides of anaphylactic mediator release. *Prostaglandins*, **16**, 179–187.

AUGSTEIN, J., FARMER, J.B., LEE, T.B., SHEARD, P. & TATTERSALL, M.L. (1973). Selective inhibitor of slow reacting substance of anaphylaxis. *Nature, New Biol.*, **245**, 215–217.

BRINK, C., GRIMAUD, C., GUILLOT, C. & OREHEK, J. (1980). The interaction between indomethacin and contractile agents on human isolated airway muscle. *Br. J. Pharmac.*, **69**, 383–388.

BURKA, J.F. & PATERSON, N.A.M. (1980). Evidence for lipoxygenase pathway involvement in allergic tracheal contraction. *Prostaglandins*, **19**, 499–515.

DAHLEN, S.E., HEDQVIST, P., HAMMARSTROM, S. & SAMUELSSON, B. (1980). Leukotrienes are potent constrictors of human bronchi. *Nature.*, **288**, 484–486.

HIGGS, G.A., COPP, F.C., DENYER, C.V., FLOWER, R.J., TATESON, J.E., VANE, J.R. & WALKER, J.M.G. (1978). Reduction of leukocyte migration by a cyclo-oxygenase and lipoxygenase inhibitor. *Proceedings of the Seventh International Congress of Pharmacology. Abstr.*, 843, p. 334. Oxford: Pergamon Press.

HITCHCOCK, M. (1980). Stimulation of the antigen-induced contraction of guinea-pig trachea and immunological release of histamine and SRS-A from sensitised guinea-pig lung by (2-isopropyl-3-indolyl)-3 pyridyl ketone (L8027) and indomethacin. *Br. J. Pharmac.*, **71**, 65–73.

OREHEK, J., DOUGLAS, J.S. & BOUHUYS, A. (1975). Contractile responses of the guinea-pig trachea *in vitro*: modification by prostaglandin synthesis-inhibiting drugs. *J. Pharmac. exp. Ther.*, **194**, 554–564.

SMEDEGARD, G., HEDQVIST, P., DAHLEN, S.E., REVENAS, B., HAMMARSTROM, S., AND SAMUELSSON, B. (1982). Leukotriene C4 affects pulmonary and cardiovascular dynamics in monkey. *Nature.*, **295**, 327–329.

(*Received July 29, 1982*)