Br. J. Pharmac. (1980), **69**, 167–169

A POSSIBLE ROLE FOR LIPOXYGENASE PRODUCTS AS REGULATORS OF AIRWAY SMOOTH MUSCLE REACTIVITY

J.J. ADCOCK & L.G. GARLAND

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

The size of guinea-pig isolated tracheal contractions induced by histamine was substantially augmented by pretreatment with the cyclo-oxygenase inhibitor, indomethacin. However, compounds which inhibit both the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism not only did not augment histamine-induced contractions of the guinea-pig trachea, but also completely reversed the increased reactivity to histamine produced by indomethacin. The SRS-A (slow reacting substance of anaphylaxis) antagonist, FPL55712, and the anti-allergic drug, cromoglycate, had no effect on the augmentation of histamine-induced contractions by indomethacin.

Introduction The bronchoconstrictor response to small doses of inhaled spasmogens, including methacholine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), is often greater in asthmatics than in healthy individuals (Mathé, Hedquist, Holmgren and Svanborg, 1973; Ryo & Townley, 1976). The mechanism of this non-specific airway hyper-reactivity has yet to be clearly defined. Relevant to this phenomenon is the observation by Orehek, Douglas & Bouhuys (1975) that the size of guinea-pig tracheal contractions induced by histamine and several other spasmogens is increased by pretreatment with indomethacin. These authors suggested that indomethacin, by inhibiting the cyclo-oxygenase pathway of arachidonic acid metabolism, may reduce the effect of a relaxant prostaglandin such as PGE_2. The present study was carried out to investigate whether augmentation of histamine-induced contractions of guinea-pig trachea by indomethacin might be due to diversion of arachidonic acid to lipoxygenase products. To investigate this hypothesis, we made use of compounds that inhibit both cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism, two such compounds being referred to as CLI, 3-amino-1-[*p*-(chlorophenyl)]-2-pyrazoline (Adcock, Garland, Moncada & Salmon, 1978b) and BW755C, 3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline (Higgs, Copp, Denyer, Flower, Tateson, Vane & Walker, 1978).

Method Male Porcellus guinea-pigs (250 to 300 g) were killed by a blow to the neck, the trachea was rapidly removed and placed in Tyrode solution of the following composition (mM): $NaCl_2$ 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. It was then cleaned of extraneous tissue, cut longitudinally through the cartilage to form a flat sheet and transverse cuts made to form a strip of tissue which was then suspended in an organ bath and superfused at a rate of 3 ml/min with Tyrode solution maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Responses were measured isotonically with a Harvard heart/smooth muscle transducer and recorded on a Rikadenki 4-channel desk-top pen recorder. Each tracheal strip was then equilibrated under an initial tension of 6 g for 1.5 h; the load was reduced to 3 g just before the start of the experiment. At least two cumulative dose-response curves for histamine were obtained before the infusion of test compound(s) which was usually for 20 min before and during the next histamine dose-response curve. Responses were expressed as percentages of the maximum control response.

Results Indomethacin (1 μg/ml) substantially augmented the histamine-induced contractions (Figure 1a) and this augmentation persisted for at least 2 h after its superfusion had ceased. Over the range of 0.1 ng/ml to 100 μg/ml indomethacin, the dose-response curve was bell-shaped, maximum histamine responses in 16 preparations being approximately doubled by indomethacin at concentrations between 10 ng/ml to 10 μg/ml. At higher concentrations the effect of indomethacin decreased in a dose-related manner, maximum responses to histamine becoming less than control (before treatment) when indomethacin was used at concentrations greater than 50 μg/ml (five preparations). In contrast to its enhancing effect, this inhibition by indomethacin was completely reversed by washing the tissue for 20 min.

A number of phenyl pyrazolines have recently been described as mixed cyclo-oxygenase/lipoxygenase inhibitors, the two compounds used in this study were

0007-1188/80/060167-03 $01.00

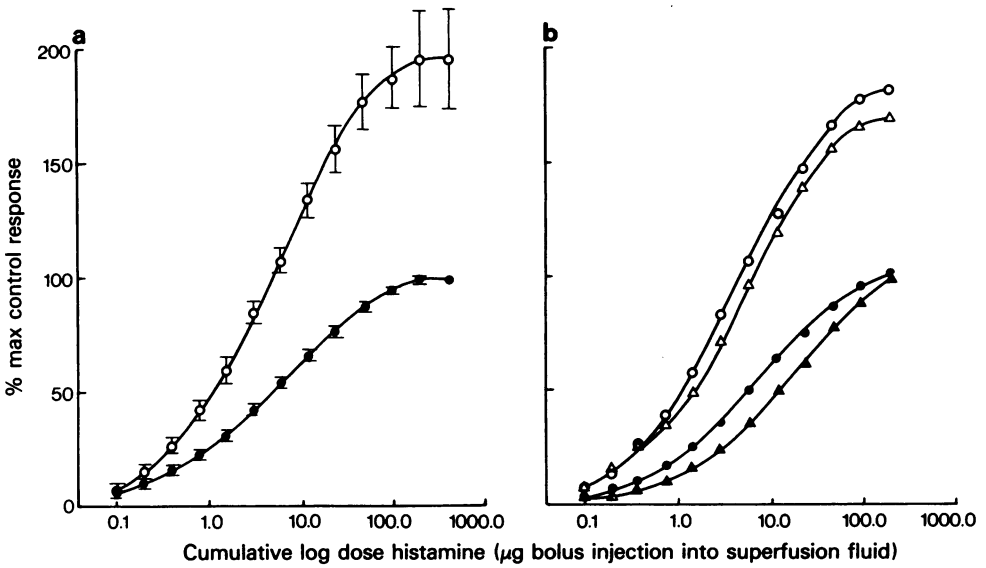

Figure 1 Histamine-induced contractions of guinea-pig trachea expressed as cumulative log dose-response curves either before (●; $n = 20$ in a, 2 in b) or after (○; $n = 7$ in a, 2 in b) treatment with indomethacin (1.0 μg/ml in a; 0.5 μg/ml in b). In (b) histamine responses after 20 min superfusion of indomethacin concomitantly with CLI (40 μg/ml) on the same preparation are shown by (▲) ($n = 2$). Following removal of CLI, indomethacin (0.5 μg/ml) superfusion was continued for a further 20 min (△; $n = 2$). Responses are expressed as percentages of the maximal control response and vertical lines show s.e. mean.

CLI (Adcock *et al.*, 1978b) and BW755C (Higgs *et al.*, 1978). When either of these inhibitors was superfused over the trachea at concentrations between 10 μg/ml to 40 μg/ml for 20 min before and during a histamine dose-response curve, the control histamine dose-response curve remained almost unchanged (results not illustrated). However, when superfused concomitantly with indomethacin (0.5 μg/ml), CLI (40 μg/ml) reduced the augmented histamine responses to control values. This effect of CLI was reversible. When superfusion of CLI was stopped but indomethacin treatment continued for a further 20 min histamine responses were again augmented (Figure 1b). Similar results (not illustrated) were obtained in two experiments with compound BW755C. These results with the mixed cyclo-oxygenase/lipoxygenase inhibitors suggest that indomethacin exerts its action not by removing the effect of a relaxant prostaglandin from the cyclo-oxygenase pathway, but by diverting arachidonic acid metabolism through the lipoxygenase pathway, a product of which might augment histamine-induced contractions.

Since it has been suggested that a product of the lipoxygenase pathway, leukotriene-C, may have slow reacting substance of anaphylaxis (SRS-A) like activity (Murphy, Hammarström & Samuelsson, 1979) we investigated the action of the SRS-A antagonist, FPL55712 sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (Augstein, Farmer, Lee, Sheard & Tattersall, 1973) against histamine contractions previously augmented by indomethacin. In two preliminary experiments, FPL55712 at a concentration of 0.5 μg/ml completely antagonized SRS-A-induced contractions of the guinea-pig trachea while leaving histamine contractions unchanged. However, the same concentration of FPL55712 neither prevented nor reversed indomethacin-augmentation of histamine contactions. Indomethacin (0.5 μg/ml) increased the maximum histamine contraction to 153 ± 8% of control (mean ± s.e. mean; $n = 7$) in untreated preparations and to 164 ± 12% of control (mean ± s.e. mean; $n = 3$) in tissues pretreated with FPL55712 (0.5 μg/ml); these values were not significantly different by t test. In three separate experiments where indomethacin (0.5 μg/ml) had already augmented maximum histamine contractions to 161 ± 15% of control, subsequent superfusion of FPL 55712 (0.5 μg/ml) left histamine responses unchanged at 159 ± 13% of control (mean ± s.e. mean). In addi-

tion, we have tested the anti-allergic drug, cromoglycate, in this system to assess whether any part of its effect in asthmatics could be attributed to an inhibition of airway hyper-reactivity associated with lipoxygenase products. However, cromoglycate (50 μg/ml) neither prevented nor reversed the augmentation of histamine contractions by indomethacin, 0.5 μg/ml (2 separate experiments, results not illustrated).

Discussion The present results confirm and extend previous observations that indomethacin augments histamine contractions of guinea-pig tracheal smooth muscle. From our results with mixed cyclo-oxygenase/lipoxygenase inhibitors we conclude that this increased responsiveness may be attributed to an augmenting effect of lipoxygenase product(s) formed in greater quantity by diversion of arachidonic acid metabolism after cyclo-oxygenase inhibition. Of the several lipoxygenase products that might be responsible we appear to have ruled out leukotriene-C since high concentrations of FPL55712 did not prevent or reverse augmentation of responses by indomethacin. Thus, it may be concluded that lipoxygenase products other than leukotriene-C may have a pathological role in asthma. The present results point to them having a role in non-specific airway hyper-reactivity while previous experiments on perfused guinea-pig lungs (Adcock, Garland, Moncada & Salmon, 1978a; Adcock *et al.*, 1978b) suggested an action on allergic mechanisms.

References

ADCOCK, J.J., GARLAND, L.G., MONCADA, S. & SALMON, J.A. (1978a) Enhancement of anaphylactic mediator release from guinea-pig perfused lungs by fatty acid hydroperoxides. *Prostaglandins*, **16**, 163–177.

ADCOCK, J.J., GARLAND, L.G., MONCADA, S. & SALMON, J.A. (1978b) 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.

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.

MATHÉ, A.A., HEDQUIST, P., HOLMGREN, A. & SVANBORG, N. (1973) Bronchial hyper-reactivity to Prostaglandin $F_{2\alpha}$ and histamine in patients with asthma. *Br. med. J.*, **1**, 193–196.

MURPHY, R.C., HAMMARSTRÖM, S. & SAMUELSSON, B. (1979). Leukotriene C: a slow reacting substance from murine mastocytoma cells. *Proc. natn. Acad. Sci., U.S.A.*, **76**, 4275–4279.

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.

RYO, U.J. & TOWNLEY, R.G. (1976). Comparison of respiratory and cardiovascular effects of isoprotenerol, propranolol and practolol in asthmatic and normal subjects. *J. Allergy Clin. Immun.*, **57**, 12–24.

(*Received January 14, 1980.*)