Br. J. Pharmac. (1985), **85**, 421–425

Pharmacological modulation of responses of guinea-pig airways contracted with arachidonic acid

John F. Burka

Department of Pharmacology, University of Alberta, 9–70 Medical Sciences Building, Edmonton, Alberta, Canada T6G 2H7

1 Arachidonic acid (AA) was used to induce contractions of guinea-pig tracheal and lung parenchymal preparations in the presence of indomethacin.

2 Prior addition of FPL55712, nordihydroguaiaretic acid (NDGA), piriprost, benoxaprofen or nafazatrom, in order of potency, inhibited AA-induced contractions of trachea. Higher concentrations (2–3 fold) were necessary to inhibit contractions of parenchyma.

3 FPL55712 and piriprost appeared to act as pharmacological antagonists of leukotrienes because they rapidly reduced the tone of the airways established by AA.

4 Administration of exogenous AA to indomethacin-treated trachea appears to be a good model to examine leukotriene receptor antagonists and inhibitors of the lipoxygenase pathway.

Introduction

Arachidonic acid (AA) is metabolized via the cyclo-oxygenase and lipoxygenase pathways to yield prostaglandins and leukotrienes, respectively. On addition of exogenous AA, the trachea converts AA primarily to prostaglandin E_2 (PGE_2), a bronchodilator, and leukotriene C_4 (LTC_4) and LTD_4, both bronchoconstrictors (Burka *et al.*, 1981; Burka & Saad, 1984a), and the lung parenchymal strip produces thromboxane A_2 (TXA_2), a bronchoconstrictor cyclo-oxygenase product, and LTC_4 and LTD_4 (Mitchell & Denborough, 1980; Burka & Saad, 1984a). Inhibition of the cyclo-oxygenase pathway with indomethacin blocks the synthesis of PGE_2 and TXA_2 but the leukotrienes are still produced (Burka & Saad, 1984a). There does not appear to be any increased synthesis of leukotrienes as a consequence of blocking the cyclo-oxygenase pathway (Burka & Saad, 1984b).

Thus it appears that AA-induced contraction of indomethacin-treated airways smooth muscle would be a direct consequence of leukotriene synthesis. Therefore, this model was used to examine the inhibitory properties of a series of putative 5-lipoxygenase inhibitors. These included nafazatrom (Mardin & Busse, 1983), 3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline (BW755C) (Higgs *et al.*, 1979), nordihydroguaiaretic acid (NDGA) (Tappel *et al.*, 1953), benoxaprofen (Cashin *et al.*, 1977), 6,9-deoxy-6,9-N-phenylimino-$\Delta^{6,8}$-PGI_1 (piriprost) (Bach *et al.*, 1982) and diethylcarbamazine citrate (DECC) (Harned *et al.*, 1948). In addition, sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy-propoxy]-4-oxo-8-propyl-4H-benzopyran-2-carboxylate (FPL-55712) (Augstein, *et al.*, 1973) and piriprost were examined as agents which block peptidoleukotriene receptors in the airways (Sheard *et al.*, 1977; Bach *et al.*, 1982; Jones *et al.*, 1983).

Methods

Male English short-hair guinea-pigs (200–250 g) (Connaught Laboratories, Toronto, Ontario) were sensitized with ovalbumin (OA), 100 mg subcutaneously and 100 mg intraperitoneally. Lungs and trachea were removed 3–4 weeks later. The trachea was spirally cut (Constantine, 1965), divided into four segments and each suspended under 1 g tension in 10 ml organ baths containing Krebs-Henseleit solution (KHS) maintained at 37°C and aerated with 95% O_2 plus 5% CO_2. Parenchymal strips were carefully cut from the distal edges of the lung lobes (Lulich *et al.*, 1979) and suspended under 500 mg tension in 10 ml organ baths as described above.

Contractions of the tracheal spirals and parenchymal strips were measured isotonically using rotary motion transducers (Type 386 heart/smooth muscle transducers; Ealing Scientific, St Laurent, Quebec). Tissues were incubated for 1 h before use. Constant

maximal contractions to histamine (10^{-4} M) were obtained before modulatory drugs were administered.

Indomethacin (8.4×10^{-6} M) was added to the organ baths at least 30 min before challenge with AA (6.6×10^{-5} M). FPL55712 and the putative lipoxygenase inhibitors were also added to the organ baths 30 min before challenge, except piriprost which was added 1 min before challenge. In one series of experiments FPL55712, piriprost, and nafazatrom were added at fixed time points following challenge of the tissues with AA.

Analysis and statistical evaluation of results

The data are presented as the mean ± s.e.mean. Responses in the figures are expressed as a percentage of the maximum response obtained with histamine on each tissue in the absence of any modulatory treatment or drug. The areas under the contraction curves over a fixed time period, as recorded on a linear chart recorder, were calculated on a computer programme. Test and control curves were compared and analysed for significance using Students *t* test for paired data. A minimum of 4 animals was used for each experiment.

Drugs

Histamine dihydrochloride, ovalbumin (grade II for sensitization), nordihydroguaiaretic acid, diethylcarbamazine citrate, calcium ionophore A23187 and arachidonic acid (99% pure) were purchased from Sigma (St Louis, MO). I am grateful for the kind gifts of the following agents: piriprost (Dr M.K. Bach, Upjohn, Kalamazoo, MI), nafazatrom (Dr A. Scriabine, Miles, New Haven, CN), BW755C (Dr P.J. McHale, Wellcome, Beckenham, Kent), FPL55712 (Mr P. Sheard, Fisons, Loughborough, Leics.), benoxaprofen (Dr R. Thompson, Dept. of Medicine, Rheumatology Div., University of Alberta) and indomethacin (Dr W. Dorian, Merck-Frosst, Pointe Claire-Dorval, Quebec).

Indomethacin (1 mg ml^{-1}) and piriprost (10^{-2} M) were dissolved in 1 M Tris buffer (pH 8.5), diluted in KHS, and kept on ice. Arachidonic acid (2 mg ml^{-1}) was dissolved in 1 M Tris buffer (pH 8.4), diluted in water and kept frozen in the dark at − 5°C until used. NDGA (10^{-2} M) was dissolved in distilled water and a few drops of NaOH (1 N) were added to make a salt. Nafazatrom (10 mg ml^{-1}) and benoxaprofen (10^{-1} M) were dissolved in dimethyl sulphoxide (DMSO). FPL55712 (10^{-2} M) and BW755C (10^{-1} M) were dissolved in distilled water and diluted with KHS. Diethylcarbamazine citrate (10^{-1} M) was dissolved in KHS. The vehicles (ethanol, Tris buffer or DMSO) did not affect tissue responses at the concentrations used.

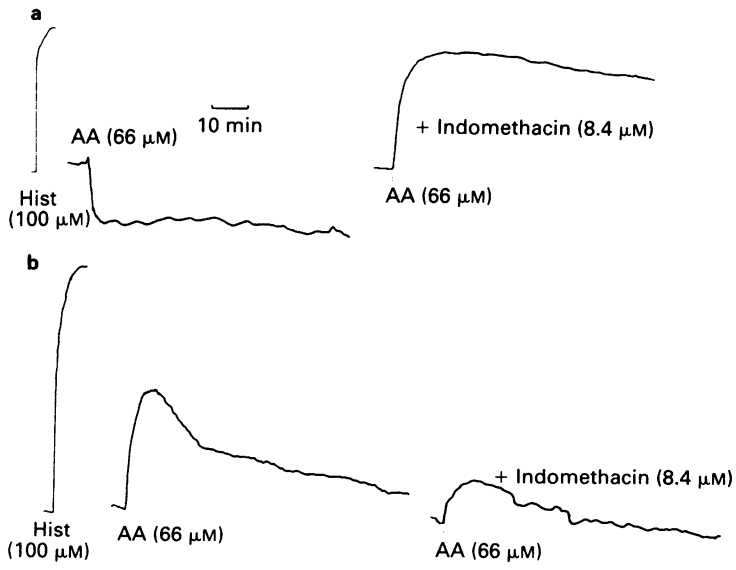

Figure 1 Representative tracings of a tracheal spiral (a) and a parenchymal strip (b) in the absence and presence of indomethacin (8.4 μM) when challenged with arachidonic acid (AA: 66 μM). A contraction obtained with histamine (100 μM) is shown for reference purposes. In the absence of indomethacin, this particular tracheal spiral relaxed when AA was added to the organ bath.

Table 1 The effects of putative inhibitors of the lipoxygenase pathway and peptidoleukotriene receptor antagonists on contractions induced by arachidonic acid on indomethacin-treated airways

	Trachea	*Parenchyma*
Nafazatrom		
3.7×10^{-5} M	81.7 ± 14.0	246.6 ± 147.6
1.2×10^{-4} M	25.0 ± 10.6*	202.2 ± 97.9
3.7×10^{-4} M	0.2 ± 0.1*	38.1 ± 37.7*
NDGA		
10^{-6} M	92.7 ± 9.5	85.0 ± 23.3
10^{-5} M	60.7 ± 10.2*	81.3 ± 13.9
10^{-4} M	25.0 ± 6.3*	32.1 ± 10.9*
Piriprost		
10^{-6} M	81.3 ± 11.0	108.2 ± 17.5
10^{-5} M	72.3 ± 27.4	69.1 ± 45.7
10^{-4} M	13.6 ± 7.0*	46.5 ± 17.0*
Benoxaprofen		
10^{-6} M	115.8 ± 22.5	100.0 ± 40.0
10^{-5} M	70.6 ± 9.5*	133.2 ± 41.9
10^{-4} M	11.7 ± 6.9*	32.2 ± 9.9*
FPL55712		
10^{-6} M	65.1 ± 18.3	121.6 ± 13.5
3×10^{-6} M	43.7 ± 10.1*	60.5 ± 22.4
10^{-5} M	18.4 ± 10.1*	34.0 ± 13.2*

Values are expressed as the mean % (± s.e.mean) of control of the areas under the contraction curves.
NDGA = nordihydroguaiaretic acid.
* $P < 0.05$.

Results

Effects of drugs on contractions induced by arachidonic acid

An efficient way of examining inhibitors of the lipoxygenase pathway or peptidoleukotriene receptor antagonists in our model was to challenge indomethacin-treated tissues with exogenous arachidonic acid. In the absence of indomethacin, AA either relaxed trachea or had no effect. In the presence of indomethacin, AA consistently contracted trachea (Figure 1a). Parenchymal contractions induced by AA were reduced in the presence of indomethacin (Figure 1b). This is in agreement with the findings of Mitchell & Denborough (1980) and Mitchell (1982). The reduced parenchymal contractions caused some difficulty for analysis because low values in relation to the maximum response to histamine increased the degree of variance and the absolute degree of enhancement when this occurred.

The peptidoleukotriene receptor antagonist, FPL55712, was ten times more potent in inhibiting AA-induced contractions of trachea than any of the inhibitors of the lipoxygenase pathway (Table 1). The IC_{50} for FPL55712 was approximately 2×10^{-6} M whereas the IC_{50}s for NDGA, benoxaprofen and piriprost were in the range of $2-3 \times 10^{-5}$ M, and that for nafazatrom was 5×10^{-5} M. DECC and BW755C did not modify AA-induced contractions of either trachea or parenchyma pretreated with indomethacin (data not shown).

Parenchymal contractions were inhibited by FPL55712, NDGA, benoxaprofen, piriprost and nafazatrom in the same order of potency as on the trachea. However, the potency of the agents was about 2–3 fold less on parenchyma than on trachea. The IC_{50} for FPL55712 was approximately 5×10^{-6} M; the IC_{50}s for NDGA, piriprost, and benoxaprofen were in the range of $4-9 \times 10^{-5}$ M; and that for nafazatrom was 3×10^{-4} M.

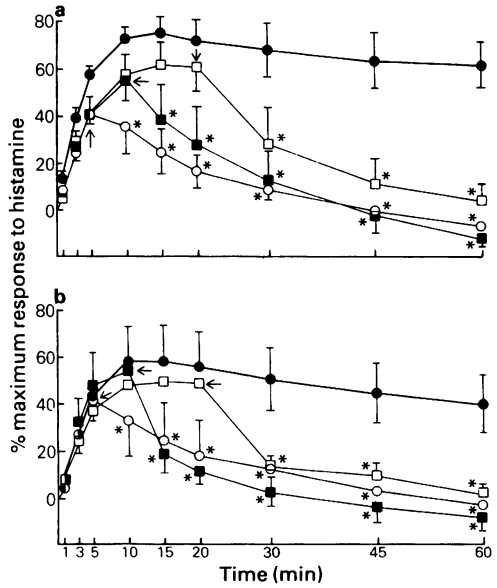

Figure 2 The contractile responses of indomethacin-treated tracheal spirals when challenged with arachidonic acid (AA: 66 μM). The arrows indicate the time points when (a) FPL55712 (10^{-5} M) or (b) piriprost (10^{-4} M) was added to the organ bath. The drugs were administered 5 (○), 10 (■) and 15 (□) min following challenge of the tissue with AA. Vertical lines indicate s.e.mean. The results are the mean of 4 experiments for FPL55712 and 3 experiments for piriprost. * $P < 0.05$, significantly different from contractions of paired trachea where FPL55712 or piriprost was not added (●).

The effects of drug addition following challenge

Drugs were administered to the organ baths 5, 10 or 20 min following challenge of the tissues with AA. FPL55712 (10^{-5} M) and piriprost (10^{-4} M) caused an immediate reduction in tracheal tone induced by AA (Figure 2). Nafazatrom (1.2×10^{-4} M) did not significantly affect AA-induced contractions when added post-challenge, as measured by the area under the contractile curve following drug administration. However, there appeared to be a trend to inhibition developing with time. At 60 min post-AA challenge, nafazatrom, which had been added 5 min post-challenge, reduced the control response by $66.0 \pm 16.3\%$.

Discussion

A direct method for looking at the role of AA in the contraction of airways was to administer AA directly to the tissues. AA was examined in the presence of indomethacin so that the possibility of AA being metabolized via the cyclo-oxygenase pathway would be eliminated. AA-induced contractions develop slowly and plateau in 10–15 min. Thus the area under the curve could be used to analyse AA-induced contractions. Indomethacin enhanced AA-induced contractions of trachea, but reduced those of parenchyma, in agreement with the results of Mitchell & Denborough (1980) and Mitchell (1982).

We had previously shown that the administration of AA to trachea or lung parenchyma resulted in the release of LTC_4 and LTD_4 (Burka & Saad, 1984a). Thus it was assumed that inhibition of AA-induced contractions of indomethacin-treated airways was an index of inhibition of leukotriene synthesis. FPL55712, NDGA, piriprost, benoxaprofen and nafazatrom, in order of potency, inhibited AA-induced contractions of trachea and 2–3 fold higher concentrations were necessary to inhibit contractions of parenchyma. This may be due to the fact that the parenchymal strip is a thicker and more heterogeneous preparation than the tracheal spiral and penetration to sites of action may be more difficult. A comparison of the effects of nafazatrom, piriprost and FPL55712 given following challenge suggests that piriprost and FPL55712 act as pharmacological antagonists of the contraction of airways because they rapidly reduced tracheal tone. It is probable that both these substances were antagonizing leukotriene receptors since piriprost and FPL55712 have been shown to be leukotriene receptor antagonists in the guinea-pig ileum and airways, respectively (Sheard *et al.*, 1977; Bach *et al.*, 1982). In contrast, the inhibition with nafazatrom took time to develop, which agrees with its reported ability to inhibit 5-lipoxygenase (Mardin & Busse, 1983). Piriprost may also have been inhibiting leukotriene biosynthesis, as it does in rat mononuclear cells (Bach *et al.*, 1982), although this must still be confirmed in airways tissue by measuring leukotriene release. Although not tested post-challenge in this study, we assumed that benoxaprofen and NDGA were inhibiting leukotriene biosynthesis because they have been shown to inhibit leukotriene release from chopped lung (Morris *et al.*, 1980; Dawson *et al.*, 1982).

BW755C (up to 10^{-4} M) and DECC (up to 10^{-3} M) did not modify AA-induced contractions of either trachea or parenchyma pretreated with indomethacin, suggesting that the compounds had no effect on the synthesis of lipoxygenase products in guinea-pig airways. This is in contrast to a study showing that BW755C inhibits antigen-induced leukotriene release from sensitized guinea-pig chopped lung (Piper & Temple, 1981) and one where BW755C reversed contractions induced by AA in the presence of indomethacin (Mitchell, 1982). The reason for the discrepancy is not clear but may result from differences in methodology. Our results are based on one administration of AA, whereas Mitchell (1982) used repeated AA administrations which may result in the mobilization of AA from different pools. Morris *et al.* (1980) have suggested that exogenous AA is incorporated into lung phospholipids and causes displacement of endogenous AA or related fatty acids which are then metabolized by a lipoxygenase at the site of action.

The above results all support the role of peptidoleukotrienes in AA-induced contractions of the airways and indicate that administration of exogenous AA to indomethacin-treated airways is a good model to examine modulators of leukotriene synthesis and receptor site antagonists in a tissue.

This work was supported by grants from the Medical Research Council of Canada, the Alberta Lung Association, and the Alberta Heritage Foundation for Medical Research. I am grateful for the excellent technical assistance of Lillian Kwan-Yeung and the secretarial assistance of Jacquie Tucker.

References

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.

BACH, M.K., BRASHLER, J.R., SMITH, H.W., FITZPATRICK, F.A., SUN, F.F. & McGUIRE, J.C. (1982). 6,9-Deepoxy-6,9-(phenylimino)-$\Delta^{6,8}$-prostaglandin I_1, (U-60,257), a new inhibitor of leukotriene C and D synthesis: *in vitro* studies. *Prostaglandins*, **23,** 759–771.

BURKA, J.F., ALI, M., McDONALD, J.W.D. & PATERSON, N.A.M. (1981). Immunological and non-immunological synthesis and release of prostaglandins and thromboxanes from isolated guinea-pig trachea. *Prostaglandins*, **22,** 683–691.

BURKA, J.F. & SAAD, M.H. (1984a). Mediators of arachidonic acid-induced contractions of indomethacin-treated guinea-pig airways: leukotrienes C_4 and D_4. *Br. J. Pharmac.*, **81,** 465–473.

BURKA, J.F. & SAAD, M.H. (1984b). Metabolism of arachidonic acid by 5-lipoxygenase in guinea-pig lung. *Prostaglandins*, **28,** 609–610.

CASHIN, C.H., DAWSON, W. & KITCHEN, E.A. (1977). The pharmacology of benoxaprofen (2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid), LRCL3794 a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthesis. *J. Pharm. Pharmac.*, **29,** 330–336.

CONSTANTINE, J.W. (1965). The spirally cut tracheal strip preparation. *J. Pharm. Pharmac.*, **17,** 384–385.

DAWSON, W., BOOT, J.R., HARVEY, J. & WALKER, J.R. (1982). The pharmacology of benoxaprofen with particular reference to effects on lipoxygenase product formation. *Eur. J. Rheum. Inflam.*, **5,** 61–68.

HARNED, B.K., CUNNINGHAM, R.W., HALLIDAY, S., VESSEY, R.E., YUDA, N.N., CLARK, M.C. & SUBBAROW, Y. (1948). Some toxicological and pharmacological properties of l-diethylcarbamyl-4-methylpiperazine hydrochloride, Hetrazan. *Ann. New York Acad. Sci.*, **50,** 141–160.

HIGGS, G.A., FLOWER, R.J. & VANE, J.R. (1979). A new approach to anti-inflammatory drugs. *Biochem. Pharmac.*, **28,** 1959–1961.

JONES, T., DENIS, D., HALL, R. & ETHIER, D. (1983). Pharmacological study of the effects of leukotrienes C_4, D_4, E_4 & F_4 on guinea pig trachealis: interaction with FPL55712. *Prostaglandins*, **26,** 833–843.

LULICH, K.M., PAPADIMITRIOU, J.M. & PATERSON, J.W. (1979). The isolated lung strip and single open tracheal ring: a convenient combination for characterizing Schultz-Dale anaphylactic contractions in the peripheral and central airways of the guinea-pig. *Clin. exp. Pharmac. Physiol.*, **6,** 625–629.

MARDIN, M. & BUSSE, W.D. (1983). Effect of nafazatrom on the lipoxygenase pathways in PMN leukocytes and RBL-1 cells. In *Leukotrienes and other Lipoxygenase Products.* ed. Piper, P.J. pp. 263–274. Chichester: Research Studies Press.

MITCHELL, H.W. (1982). Effect of ETYA and BW755C on arachidonate-induced contractions in the guinea-pig isolated trachea. *Br. J. Pharmac.*, **76,** 527–529.

MITCHELL, H.W. & DENBOROUGH, M.A. (1980). The metabolism of arachidonic acid in the isolated tracheal and lung strip preparations of guinea-pigs. *Lung*, **158,** 121–129.

MORRIS, H.R., PIPER, P.J., TAYLOR, G.W. & TIPPINS, J.R. (1980). The role of arachidonate lipoxygenase in the release of SRS-A from guinea-pig chopped lung. *Prostaglandins*, **19,** 371–383.

PIPER, P.J. & TEMPLE, D.M. (1981). The effect of lipoxygenase inhibitors and diethylcarbamazine on the immunological release of slow reacting substance of anaphylaxis (SRS-A) from guinea-pig chopped lung. *J. Pharm. Pharmac.*, **33,** 384–386.

SHEARD, P., LEE, T.B. & TATTERSALL, M.L. (1977). Further studies on the SRS-A antagonist FPL55712. *Monogr. Allergy*, **12,** 245–249.

TAPPEL, A.L., LUNDBERG, W.O. & BOYER, P.D. (1953). Effect of temperature and anti-oxidants upon lipoxidase-catalyzed oxidation of sodium linoleate. *Arch. Biochem. Biophys.*, **42,** 293–304.

(Received September 25, 1984.
Revised January 15, 1985.
Accepted February 5, 1985.)