Br. J. Pharmac. (1982) **77**, 701–705

THE EFFECT OF MIXED INHIBITORS OF CYCLO-OXYGENASE AND LIPOXYGENASE ON THE INDOMETHACIN-INDUCED HYPER-REACTIVITY IN THE ISOLATED TRACHEA OF THE PIG

H.W. MITCHELL

Department of Physiology, University of Sheffield, Sheffield S10 2TN

1 Indomethacin enhances muscle contraction to histamine in the pig isolated trachea. The effect of three cyclo-oxygenase/lipoxygenase inhibitors on these potentiated histamine-induced responses was examined.

2 At the higher concentrations BW 755c (113–226 μM), 5, 8, 11, 14-eicosatetraynoic acid (ETYA, 10–100 μM) and phenidone (5–500 μM) abolished the effect of indomethacin on tracheal contractions to histamine (100 μM).

3 Each of the three inhibitors also reduced the slope of the histamine concentration-response curve obtained in the presence of indomethacin. However, the sensitivity (pD_2) of the smooth muscle to histamine was not affected by BW 755c, ETYA or phenidone.

4 The results provide further support for a lipoxygenase-dependent component of indomethacin-induced hyper-reactivity in the pig trachea.

Introduction

Recent observations suggest that tracheal smooth muscle contraction, in response to non-immunological stimuli can be enhanced by a lipoxygenase-dependent mechanism. This is seen when the cyclo-oxygenase pathway for arachidonic acid is blocked by indomethacin or other non-steroidal anti-inflammatory drugs. The mechanisms and precise mediators of this presumed lipoxygenase pathway are unknown. In the guinea-pig isolated trachea, Adcock & Garland (1980) have found that histamine-induced contractions are potentiated by indomethacin and they showed that this effect is blocked by two pyrazoline drugs which have mixed anticyclo-oxygenase and antilipoxygenase activity. Also in the guinea-pig trachea, arachidonate-induced contractions (in the presence of indomethacin) are blocked by the mixed inhibitors 5, 8, 11, 14-eicosatetraynoic acid (ETYA) and BW 755c (Everitt, Bentley, Spiegel & Porter, 1979; Mitchell, 1982a). BW 755c was one of the drugs used by Adcock & Garland (1980). Further evidence for interaction between arachidonic acid derived metabolites and muscle contraction has been obtained in the pig isolated trachea. Mitchell (1982b) showed that drug-induced responses are inhibited by mepacrine, which inhibits phospholipase A_2, and the potentiating effect of indomethacin on drug contractions (hyper-reactivity) is reversed by ETYA. These observations suggest indirectly that airways smooth muscle tone can be regulated by arachidonic acid-derived lipoxygenase products. In the pig trachea the mechanisms underlying these effects appear to be relatively specific for contractions evoked by histamine. In this paper further information is presented on the mechanism underlying the indomethacin-induced hyper-reactivity in the pig trachea. The effects of some mixed antilipoxygenase and anticyclo-oxygenase drugs have been more fully studied and the results provide strong evidence for lipoxygenase involvement in muscle contraction in this preparation.

Methods

Pig tracheal smooth muscle was prepared for recording isometric tension as previously described (Mitchell, 1982b). In each experiment four preparations were arranged in organ baths containing Krebs solution at 37°C. The composition of the Krebs solution was (mM): NaCl 121, KCl 5.4, $MgSO_4.7H_20$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15, D-glucose 11.5 and $CaCl_2.2H_2O$ 2.5. The tissues were initially stretched to a resting tension of about 2 g then they were stimulated with acetylcholine (3 μM) until consistent contractions were obtained. In some experiments a single control response was also obtained to histamine (100 μM). Depending on the particular experiment, pairs of tracheae were then incubated in 0.01% v/v ethanol (controls to test for sensitivity

0007-1188/82/120701-05 $01.00

changes), indomethacin 1 μM or indomethacin plus BW 755c, ETYA or phenidone for 1 h before again measuring the muscle contraction to histamine and acetylcholine. In the experiments shown in Figures 1, 2 and 3 and Table 2, concentration-response curves to histamine were obtained in matched pairs of tracheae by a cumulative dosing schedule. Results shown are mean ± s.e.mean except for those shown in Table 2. Statistical analysis was by the paired *t* test unless otherwise stated.

Drugs

The following were used: acetylcholine chloride (BDH), histamine acid phosphate (BDH), indomethacin (Sigma; made up in ethanol), BW 755c (3-amino 1-[*m*-(trifluoromethyl) phenyl]-2-pyrazoline) (Wellcome Research Laboratories), ETYA (Roche; made up in ethanol) and phenidone (1-phenyl-3-pyrazolidone) (Sigma).

Results

Effect of BW 755c, ETYA and phenidone

(a) *On contractions to single concentrations of histamine and acetylcholine* In the present study, responses to histamine (100 μM) and acetylcholine (3 μM) were potentiated by 180.9% and by 13.5% respectively by 1 μM indomethacin (see Table 1). These concentrations of acetylcholine and histamine produced about 50% of their maximum responses. The contractions were controlled and corrected for tissue sensitivity changes, as determined from parallel control experiments (see Methods) and the effect of indomethacin, thus measured, was similar to that previously described for the pig trachea (Mitchell, 1982b).

BW 755c, ETYA and phenidone all antagonized this potentiating action of indomethacin on histamine-induced contractions (Table 1). Controlled experiments were performed using two concentrations of each agonist. In the presence of the lower concentrations (113 μM, 50 μM and 5 μM respectively for BW 755c, ETYA and phenidone) indomethacin still caused significant enhancement of the histamine-induced contractions. In a further experiment (not shown in Table 1) histamine-induced contractions in the presence of indomethacin and 10 μM ETYA were potentiated by 214% ($n = 2$). However, at the higher inhibitor concentrations responses to histamine were not significantly different from control contractions obtained in the absence of indomethacin. In other words, the effect of indomethacin had been abolished by the high concentrations of BW 755c, ETYA and phenidone. In no cases were the contrac-

Table 1 Effect of indomethacin (1 μM) alone and combined with BW 755c, ETYA and phenidone on acetylcholine and histamine-induced contractions

	Indomethacin	*Indomethacin + BW 755c*		*Indomethacin + ETYA*		*Indomethacin + phenidone*	
		113 μM	226 μM	50 μM	100 μM	5 μM	100 μM
Acetylcholine (3 μM)	+13.5 ± 19.6 (8)	+6.8 ± 9.6 (6)	+3.1 ± 13.8 (6)	+4.9 ± 9.8 (4)	NT	+12.6 ± 27.4 (4)	+18.8 ± 18.9 (3)
Histamine (100 μM)	+180.9 ± 42.8** (8)	+169.9 ± 58.3* (6)	+53.3 ± 57.2 (6)	+139.2 ± 43.5* (6)	+59.2 ± 55.6 (4)	+86.5 ± 20.9* (4)	−3.5 ± 19.6 (3)

The values shown are mean ± s.e.mean percentage differences in acetylcholine and histamine-induced contractions before and after treatment with BW 755c, ETYA or phenidone. Number of preparations in parentheses. * $P < 0.05$; ** < 0.01 compared to controls (paired *t* test, see Methods). NT, not tested.

tions in response to acetylcholine modified (Table 1).

(b) *On the histamine concentration-response relationship* Pairs of tracheae, matched both by their location in the trachea and by their contractile response to acetylcholine (3 μM), were incubated in indomethacin (1 μM). One tissue in the pair was also incubated with BW 755c (113–226 μM), ETYA (10–100 μM) or phenidone (5–500 μM). Final bath concentrations of ethanol (solvent for indomethacin and ETYA) were always the same.

Tracheal preparations from different pigs varied considerably in their sensitivity (concentration producing 50% maximum effect, EC_{50}) and reactivity (slope) to histamine. In 40 preparations the pD_2 (negative log EC_{50}) values ranged from 3.60–4.90 (mean 4.02, Table 2) for histamine in the presence of indomethacin, and contractions to histamine (100 μM) ranged from 0.04–13.8 g tension. Therefore responses in each pair of tissues were normalised by expressing contractions as a percentage of the maximum contraction obtained to 1 mM histamine, in the preparation which was incubated in indomethacin. Each of the three inhibitor drugs depressed contractions to histamine (in the presence of indomethacin) and shifted the concentration-response curves to the right, in a dose-dependent manner (Figures 1, 2 and 3). Low concentrations of BW 755c (113 μM) and ETYA (50 μM) displaced the histamine concentration-response relationship to the right. It was notable that phenidone (5 μM) appeared to augment the histamine-induced responses, but this apparent trend was not confirmed by the experiments shown in Table 1. Higher concentrations of BW 755c, ETYA and phenidone all reduced the reactivity of the trachea to histamine i.e. they reduced the slope of the histamine concentration-response curve. In terms of proportional inhibition the antagonists appeared to have as much inhibitory effect on the histamine-induced responses at the lower end of the response curves as on the larger responses to high concentrations.

Table 2 Effects of indomethacin (1 μM) alone and combined with BW 755c, ETYA and phenidone on the sensitivity† of tracheae to histamine

		n	pD_2†(mean ± s.d.)
Indomethacin		40	4.02 ± 0.34
Indomethacin + BW 755c	113 μM	4	3.84 ± 0.13
Indomethacin + BW 755c	226 μM	6	3.73 ± 0.32
Indomethacin + ETYA	10 μM	6	3.89 ± 0.13
Indomethacin + ETYA	50 μM	4	4.06 ± 0.11
Indomethacin + ETYA	100 μM	4	3.59 ± 0.06*
Indomethacin + phenidone	5 μM	4	3.81 ± 0.14
Indomethacin + phenidone	100 μM	6	3.95 ± 0.43
Indomethacin + phenidone	500 μM	6	4.31 ± 0.59

Values shown are mean ± s.d.mean pD_2 for histamine.
†pD_2 is the negative log EC_{50} (see Results).
n, number of preparations. *$P < 0.05$ compared to indomethacin alone (unpaired *t* test).

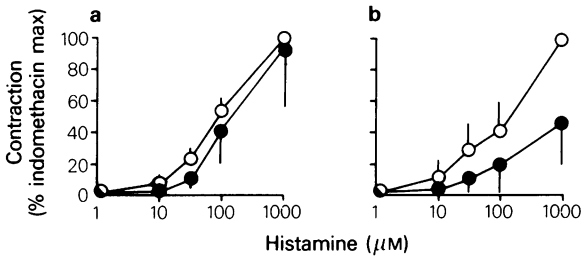

Figure 1 Histamine concentration-response curves in the presence of 1 μM indomethacin (○) and indomethacin plus BW 755c (●), 113 μM (a) and 226 μM (b). Matched pig tracheal strips were incubated in Krebs solution containing either indomethacin or indomethacin plus BW 755c. After 1 h responses to histamine were obtained. Responses in strips expressed as percentage of maximal contraction obtained to histamine in the presence of indomethacin. S.e.mean bars are shown when they are greater than the symbols. Values shown are the mean of 4 preparations.

The EC_{50} values for histamine were estimated, by eye, from individual curves. From these data mean pD_2 values were calculated and they are shown in Table 2. Except for 100 μM ETYA, none of the inhibitors significantly affected the sensitivity of tracheae to histamine. When the pD_2 for histamine in the presence of 100 μM ETYA is compared with its own control (i.e. with indomethacin alone) then the difference was not significant (3.70 ± 0.04, $n = 4$, and 3.59 ± 0.06 for indomethacin and indomethacin plus ETYA respectively). Furthermore, the pD_2 values for histamine in the presence of BW 755c, ETYA and phenidone were not significantly different from control values when likewise compared.

Discussion

The data presented here provide convincing pharmacological evidence for lipoxygenase involvement in histamine-induced contractions in isolated airways smooth muscle. The results with ETYA underscore earlier results obtained with the pig trachea (Mitchell, 1982b). In that study, single concentrations only of ETYA and histamine were tested, and the results

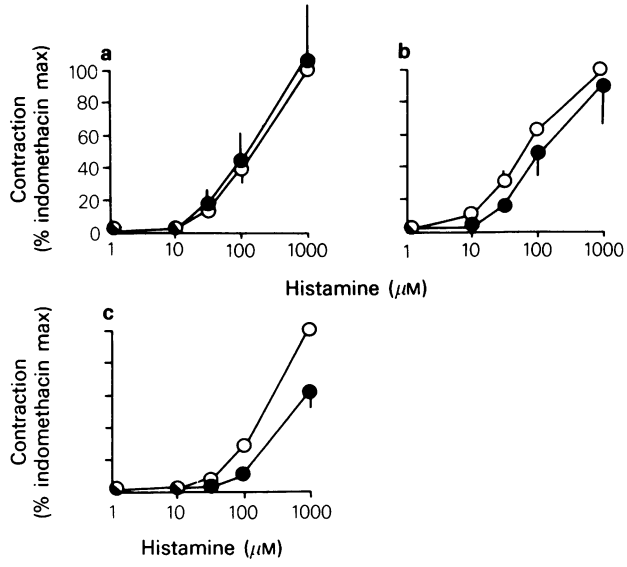

Figure 2 Histamine concentration-response curves in the presence of indomethacin 1 μM (○) and indomethacin plus ETYA (●), 10 μM (a), 50 μM (b) and 100 μM (c). Procedure as in Figure 1. Means of 4–6 preparations.

with BW 755c confirm and extend those found in guinea-pig tracheal smooth muscle (Adcock & Garland, 1980).

The effects of three inhibitors of arachidonate metabolism have been measured in smooth muscle incubated in indomethacin. These agents, BW 755c (Higgs, Flower & Vane, 1979), ETYA (Downing, Ahern & Bachta, 1970) and phenidone (Blackwell & Flower, 1978) have all been shown to inhibit the elaboration of both cyclo-oxygenase and lipoxygenase products. The concentrations chosen in the present study reflect the reported potency of these agents in inhibiting the release of prostaglandins and hydroxy acids. Two experimental protocols were used: first to evaluate quantitatively the effect of BW 755c, ETYA and phenidone on contractions to acetyl-

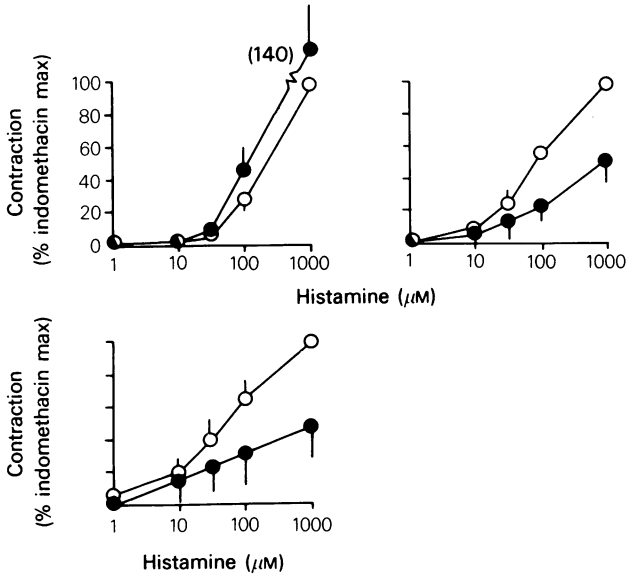

Figure 3 Histamine concentration-response curves in the presence of indomethacin 1 μM (○) and indomethacin plus phenidone (●), 5 μM (a), 100 μM (b) and 500 μM (c). Procedure as in Figure 1. Means of 4–6 preparations.

choline and histamine. The results demonstrate that BW 755c, ETYA and phenidone inhibit or abolish the increased tracheal contractions to histamine, seen in the presence of indomethacin. Second it was shown that BW 755c, ETYA and phenidone each reduced the tracheal reactivity to histamine. That is to say the slope of the histamine concentration-response curve was depressed. These results suggest that in the presence of indomethacin either excitatory prostaglandins or lipoxygenase products, which enhance muscle contraction evoked by histamine, are released. The former suggestion is very unlikely since indomethacin blocks the production of prostaglandins.

In addition to the present study and those cited above there are several reports suggesting that non-immunological contraction of airways smooth muscle may be regulated by a lipoxygenase-dependent mechanism. For example mixed inhibitors of cyclo-oxygenase and lipoxygenase inhibit arachidonate-induced contractions in guinea-pig lung strips (Mitchell & Denborough, 1980; Yen, 1981) and reduce or eliminate arachidonate-induced contractions in the presence of indomethacin in the guinea-pig trachea (Everitt *et al.*, 1979; Mitchell, 1982a). Whether lipoxygenase-dependent muscle contraction is the result of the release of a 'contractile' metabolite, or whether it is due to lipoxygenase involvement in the contractile process to histamine itself is not known. There are clearly some differences between using high concentrations of substrate (arachidonic acid) in order to stimulate lipoxygenase activity and histamine-induced muscle contraction. Presumably histamine must first mobilize, substrate in some way from the cell membrane. Furthermore, the nature of an excitatory lipoxygenase product if formed is unknown: contractions appear refractory to FPL 55712 and therefore there is no evidence indicating the involvement of a leukotriene (Adcock & Garland, 1980; Mitchell, 1982b). Some of these questions may be resolved by perfusion and bioassay techniques.

Finally, the potentiating effect of indomethacin has been found to vary considerably in magnitude and there was a high variance in the effects of histamine in the presence of indomethacin and BW 755c, ETYA and phenidone (see Table 2). It is possible that this may represent a wide spectrum of action of histamine in different individuals. This may also explain why higher concentrations of ETYA were required in the present study compared to earlier observations (Mitchell, 1982b). The differences in cellular regulation described in the present study in the pig may contribute to the wide variation in the pulmonary response to histamine aerosol seen in conscious animals as described by Douglas, Dennis, Ridgway & Bouhuys (1973).

Gifts of ETYA and BW 755c were generously supplied by T. Collins, Roche Products Ltd., and J.J. Adcock, Wellcome Research Laboratories respectively.

References

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.

BLACKWELL, G.J. & FLOWER, R.J. (1978). 1-Phenyl-3-pyrazolidone: an inhibitor of cyclo-oxygenase and lipoxygenase pathways in lung and platelets. *Prostaglandins*, **16,** 417–425.

DOWNING, D.T., AHERN, D.G. & BACHTA, M. (1970). Enzyme inhibition by acetylenic compounds. *Biochem. biophys. Res. Commun.*, **40,** 218–223.

DOUGLAS, J.S., DENNIS, M.W., RIDGWAY, P. & BOUHUYS, A. (1973). Airway constriction in guinea-pigs: interaction of histamine and autonomic drugs. *J. Pharmac. exp. Ther.*, **184,** 169–179.

EVERITT, B.J., BENTLEY, J.A., SPIEGEL, W.D. & PORTER, N.A. (1979). Inhibition of anaphylactic and arachidonic acid-induced contractions of guinea-pig isolated trachea by a cyclo-oxygenase and lipoxygenase inhibitor. *Pharmacologist*, **21,** 153 (abstract).

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

MITCHELL, H.W. (1982a). Effect of ETYA and BW 755c on arachidonate-induced contractions in the guinea-pig isolated trachea. *Br. J. Pharmac.*, **76,** 527–529.

MITCHELL, H.W. (1982b). The effect of inhibitors of arachidonic acid metabolism on drug-induced contractions in isolated tracheal smooth muscle of the pig. *Br. J. Pharmac.*, **75,** 129–136.

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.

YEN, S.S. (1981). Inhibition of arachidonic acid-induced contraction of guinea-pig lung strips. *Prostaglandins*, **22,** 183–194.

(Received April 21, 1982. Revised June 7, 1982.)