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EFFECT OF ETYA AND BW 755c ON ARACHIDONATE-INDUCED CONTRACTIONS IN THE GUINEA-PIG ISOLATED TRACHEA

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1 Arachidonic acid caused larger contractions in indomethacin-treated guinea-pig trachea than in control tracheae.

2 No change in contractions was obtained in tracheae treated with 5, 8, 11, 14, eicosatetraynoic acid (ETYA 10 μM).

3 ETYA (100 μM) blocked the effect of indomethacin on arachidonic acid-induced responses. Likewise BW 755c (113 μM) reversed the effect of indomethacin.

4 The results show that arachidonate lipoxygenase products may be responsible for the contractile responses seen in the presence of indomethacin.

Introduction

When the prostaglandin precursor, arachidonic acid, is perfused over preparations of guinea-pig isolated tracheal smooth muscle, it causes either weak contraction, weak relaxation or it has no measurable effect (Orehek, Douglas & Bouhuys, 1975; Lambley & Smith, 1975; Burka & Paterson, 1980; Mitchell & Denborough, 1980). The guinea-pig lung strip on the other hand, consistently contracts in response to arachidonic acid (Mitchell & Denborough, 1980). It is thought that these changes are due to the metabolism of substrate to either contractile or relaxant products of the cyclo-oxygenase pathway. However, the above authors have noted that when tracheae are relaxed by indomethacin, or other cyclo-oxygenase inhibitors, arachidonic acid then causes consistent contraction. This is most notable when control arachidonic acid-induced relaxation is converted into contraction by indomethacin. Recently it has been suggested that drug-induced tone in tracheal smooth muscle may be increased by the release of products of the arachidonic acid lipoxygenase pathway (Adcock & Garland, 1980; Mitchell, 1982). This is most apparent when the cyclo-oxygenase outlet for arachidonic acid is blocked with indomethacin. It was pertinent, therefore, to determine whether arachidonic acid-induced contraction in indomethacin-treated tracheae could be attributed to the synthesis of a lipoxygenase product.

Methods

Guinea-pigs weighing between 500–1000 g were stunned then bled-out. The trachea was removed and placed in Krebs solution (Mitchell & Denborough, 1979). Tracheal rings, 3 mm wide, were opened along the cartilage and suspended in organ baths containing gassed (95% O_2/5% CO_2) Krebs solution at 37°C. Tension was recorded with isometric strain gauges. Initially, a load of 1–2 g was placed in each tissue. In most experiments, four preparations were run simultaneously. Following equilibration control responses were obtained to arachidonic acid (100 μM). Then the tracheae were treated for 1 h with either indomethacin (1 μM), 5, 8, 11, 14, eicosatetraynoic acid (ETYA, 10 μM), indomethacin plus ETYA (10–100 μM), indomethacin plus BW 755c (3-amino-1-(*m*-(trifluoromethyl) phenyl)-2-pyrazoline) (113 μM) or 0.1% v/v ethanol (controls). Responses to arachidonic acid were then repeated in the presence of the inhibitor drug or ethanol control. Results are expressed as mean ± s.e.mean. Student's paired *t* test was used to test for significant responses.

The drugs were: indomethacin (Sigma), ETYA (Roche) and BW 755c (Wellcome Research Laboratories). The potassium salt of arachidonic acid (Sigma) was prepared by dissolving the acid in alcoholic KOH. Solutions were stored in the dark, under N_2 and at −20°C. On the day of the experiment, the solution was dried under N_2 then redissolved in Krebs solution or distilled water.

Results

Arachidonic acid (100 μM) had variable and weak effects on tracheal tone. Out of 39 preparations, arachidonic acid caused contraction in 26, relaxation in 8 and it had no effect in the remaining 5 tracheae. However, in the presence of 1 μM indomethacin, arachidonic acid-induced contractions were signific-

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antly increased (Table 1) and the relaxation responses seen in some preparations were converted into contractions. In ethanol-treated control tracheae arachidonic acid-induced responses were not significantly altered ($P > 0.05$, $n = 9$).

ETYA (10 μM) had no effect on arachidonic acid-induced responses, nor did it affect the bigger responses seen in the presence of indomethacin. However, at 100 μM, ETYA reversed the effect of indomethacin on arachidonic acid contractions. Arachidonic acid-induced contractions in indomethacin-treated tracheae were also blocked by BW 755c (113 μM). Data are shown in Table 1.

Indomethacin, ETYA and BW 755c reduced the intrinsic tone of tracheae (Table 1) whereas ethanol did not ($P > 0.05$, $n = 9$). Arachidonic acid-induced contractions in the presence of ethanol were negatively correlated with the intrinsic tone ($r = -0.82$, $n = 9$, $P < 0.01$). Thus bigger contractions were observed to arachidonic acid when the tone was low. No such correlation was apparent between intrinsic tone and contractions in indomethacin-treated preparations ($r = -0.51$, $n = 11$, $P > 0.05$).

Discussion

The results from this study confirm that indomethacin increases tracheal contraction by arachidonic acid and moreover, it can convert arachidonic acid-induced relaxation into a contraction. The mechanisms involved in this effect of indomethacin are unknown. It was suggested by Orehek *et al.* (1975) and by Lambley & Smith (1975) that the relaxed state of the tissue, caused by indomethacin, might be responsible. However, the present results are inconsistent with this suggestion. No correlation could be shown between reduction in intrinsic tone and arachidonic acid-induced contraction in the presence of indomethacin. Furthermore, Mitchell & Denborough (1980) found that tracheae which had been relaxed manually did not then contract in response to arachidonic acid. There was a significant negative correlation in the present experiments between the level of intrinsic tone and the size of arachidonic acid-induced contractions in control tracheae. This suggests that different mechanisms may be involved in the responses to arachidonic acid with and without indomethacin present.

Evidence has been recently presented suggesting that products of the lipoxygenase pathway may play a role in regulating drug-induced tone in guinea-pig (Adcock & Garland, 1980) and swine (Mitchell, 1982) tracheal smooth muscle. It has been suggested that indomethacin may encourage arachidonic acid to pass through the lipoxygenase pathway (resulting in enhanced contractions), possibly because cyclo-oxygenase is blocked. The nature of the product(s) thus formed is unknown, but it does not appear to be a leukotriene because responses are resistant to FPL 55712 (Adcock & Garland, 1980; Mitchell, 1982). A similar mechanism to this could operate in the indomethacin-treated guinea-pig trachea when arachidonic acid is given. Indeed, this was suggested by Burka & Paterson (1980) because they found that arachidonic acid did not cause contraction in the presence of the mixed cyclo-oxygenase/lipoxygenase inhibitor ETYA (Downing, Ahern & Bachta, 1970; Hamberg, 1976). Results described here confirm this finding. Furthermore, the present experiments show that ETYA also reverses the effect of indomethacin on arachidonic acid-induced responses. Relatively high concentrations of ETYA were required (100 μM). In the pig trachea 10 μM ETYA abolishes the effect of indomethacin on histamine-induced contractions (Mitchell, 1982) and arachidonic acid-induced contractions (no indomethacin present) in guinea-pig lung strips are blocked by 50 μM ETYA (Mitchell & Denborough, 1980). Unless high concentrations of ETYA (100 μM) exert unknown non-specific effects, these results suggest that products of the lipoxygenase pathway are responsible for the arachidonic acid-induced contractions following indomethacin. This is supported by the finding that the reported inhibitor of rat lung cyclo-oxygenase and lipoxygenase, BW 755c (Higgs, Copp, Denyer, Flower, Tateson, Vane & Walker, 1978) likewise reversed the effect of indomethacin; this confirmed

Table 1 The effect of indomethacin, ETYA and BW 755c on the guinea-pig isolated trachea

	n	*Change in intrinsic tone* (mg)	*Change in arachidonic acid contraction* (mg)†
Indomethacin 1 μM	11	−505.5 ± 116.6**	+287.5 ± 89.7**
ETYA 10 μM	4	−225.0 ± 63.9*	+ 31.5 ± 33.9
ETYA 10 μM + indomethacin 1 μM	7	−711.4 ± 173.6**	+251.4 ± 79.4*
ETYA 100 μM + indomethacin 1 μM	4	−535.0 ± 174.5	− 57.5 ± 16.0
BW 755c 113 μM + indomethacin 1 μM	4	−712.5 ± 51.5**	− 35.0 ± 46.3

Values shown are mean ± s.e.mean; *n*, number of tissues. *$P < 0.05$, **$P < 0.01$ (paired *t* test). †Difference between arachidonic acid-induced contraction before and after the inhibitor drug.

an earlier observation by Everitt, Bentley, Spiegel & Porter (1979). There is evidence that lipid hydroperoxides can affect lung mediator release and airways smooth muscle tone (Adcock, Garland, Moncada & Salmon, 1978; Everitt *et al.*, 1979) and their release may thus be a candidate for the mode of action of indomethacin on tracheal contractions.

There is a strong parallel between the effects of indomethacin and other cyclo-oxygenase inhibitors, on airways smooth muscle *in vitro* and the condition known as 'aspirin-induced asthma'. A clear relationship has been established between 'aspirin-induced asthma' and inhibition of prostaglandin production (see Szczeklik, Gryglewski & Czerniawska-Mysik, 1977). However, the present results and those reported elsewhere (Adcock & Garland, 1980; Mitchell, 1982) show the need for further experimentation on the possible involvement of arachidonic acid lipoxygenase products in the regulation of airways smooth muscle and 'aspirin-induced asthma'.

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