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The effects of arachidonic acid and non-steroidal anti-inflammatory drugs on intrapulmonary airways of the guinea-pig

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1 A method is described in which changes in intrapulmonary airway tone of guinea-pig isolated lungs are reflected by changes in intraluminal perfusion pressure.

2 A supramaximal dose of arachidonic acid (AA) (61μM) was found to have little on no action on baseline perfusion pressure. However, following elevation of perfusion pressure with histamine, AA caused a dose-dependent pressure decrease. This was also mimicked by prostaglandin E_1 (PGE_1) and PGE_2.

3 AA induced a reduction of histamine elevated perfusion pressure which was inhibited, dose-dependently, by several non-steroidal anti-inflammatory agents including indomethacin, phenylbutazone, aspirin, benoxaprofen, BW755C and phenidone. Their respective rank order of potency appeared to correlate with their activity against microsomal cyclo-oxygenase.

4 Indomethacin, phenylbutazone and aspirin induced augmentation of the elevated perfusion pressure due to histamine, whereas BW755C did not.

5 We suggest that the primary arachidonate metabolite present in intrapulmonary airways following histamine-induced constriction is probably a relaxant of the E series. However, our data suggest that both cyclo-oxygenase and lipoxygenase products are associated with the maintenance of airway tone.

Introduction

The effect of exogenously applied arachidonic acid (AA) on respiratory smooth muscle is complex and appears to depend on the site of the airway within the tracheobronchial tree. For example, AA induces either constriction or relaxation (or a combination of both) in relaxed and constricted tracheal chains (Mitchell & Denborough, 1980), whereas it consistently constricts parenchymal strips (Yen, 1981).

During constriction, pulmonary metabolism of AA is enhanced, but again the principal metabolite generated depends on the site of the stimulated airway; some constricted isolated tracheae release prostaglandin (PG)-like substances of the E series (Palmer *et al.*, 1973) whereas other constricted parenchymal strips release thromboxane (TX)-like substances (Gryglewski *et al.*, 1976; Mitchell & Denborough, 1980). PGE_2 is known to relax bronchial smooth muscle (Sweatman & Collier, 1968) while TXA_2 is a potent constrictor (Svensson *et al.*, 1977). The inference from such studies is that endogenous and exogenous AA is metabolized differently in 'central and peripheral airways.

There is evidence to suggest that the effect of AA on the parenchymal strip preparation may not accurately reflect its action of peripheral airways (Eyre & Mirbahar, 1981). This preparation also comprises vascular smooth muscle which is thought to contribute substantially to its contractile and metabolic profile, and possibly some non-muscular contractile elements (Goldie *et al.*, 1982).

Since the effects of AA on the intact bronchial tree remain controversial, we have attempted to determine its effects on a guinea-pig isolated lung model involving intrapulmonary airway perfusion. In addition, a number of non-steroidal anti-inflammatory drugs (NSAIDs) were assessed for their intrinsic activity on airway tone and their ability to modulate the effects of AA. Some of this work has already been presented to the British Pharmacological Society. (Clay & Fenske, 1982).

Methods

Male Dunkin-Hartley guinea-pigs (450–700 g) were anaesthetized with Valium 2.5 mg kg^{-1} and Hypnorm 1 ml kg i.m. A tracheostomy was performed and the animals exsanguinated by severing the carotid arteries. The lungs were then removed and connected via the tracheal cannula to the outflow of a perfusion apparatus. The peripheral margin of each lobe of the lungs was trimmed off to allow the perfusion fluid to escape.

Krebs solution aerated with 95% O_2 : 5% CO_2 was drawn from a reservoir at a constant rate of 5 ml min^{-1} by a peristalic pump (Watson Marlow) into a glass heating coil. A constant temperature of 37°C was maintained at the lung, and the temperature was continuously monitored using a digital probe thermometer (Ellab). A bubble trap distal to the pump head served to prevent free gas in the perfusion fluid from entering the lungs. Partial or complete occlusion of the outflow of the system resulted in the elevation of pressure between the outflow and the pump head; this was detected using a pressure transducer (Bell and Howell) distal to the bubble trap, and recorded on a heated stylus chart recorder (Ormed).

The bronchomotor effects of arachidonic acid were determined on histamine constricted and non-constricted intrapulmonary airways. Constriction of these airways was achieved by adding histamine to the Krebs solution in the reservoir. On obtaining a steady elevated baseline, the effect of bolus injections of AA on perfusion pressure was examined.

Arachidonic acid was injected into the perfusion solution just proximal to the pump head and its concentration at the lung estimated by dividing the amount injected by the dead space distal to the point of injection. The effects of increasing doses of AA (dose-interval 30 min) were examined in order to determine whether the response to AA was dose-related. Furthermore the effect of repeated administration of a supramaximal concentration of AA was determined to establish response reproducibility.

The interactions of the NSAIDs indomethacin, benoxaprofen, BW755C, phenylbutazone, aspirin, and phenidone, with AA in the histamine-constricted lung were examined. The NSAIDs were added to the reservoir 10 min after the first supramaximal bolus dose of AA and 20 min before the second bolus injection of AA. The effects of these agents on the responses to AA were determined at different doses. In addition the effects of several NSAIDs, viz. indomethacin, phenylbutazone, aspirin and BW755C on the histamine-elevated baseline alone were evaluated, in order to determine their intrinsic effects on airway tone.

Drugs

The following were used: sodium arachidonate, 90% pure (Sigma), histamine diphosphate (Sigma), indomethacin (Sigma), phenylbutazone (Sigma), aspirin (Aldrich), phenidone (Aldrich), Valium (Roche) and Hypnorm (Janssen). BW755C (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) was a gift from Burroughs Wellcome Research. The Krebs solution used had the following composition (mM): NaCl 96, KCl 4.7, $CaCl_2$ $2H_2O$ 2.4, $MgSO_4$ $7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 12. These constituents were purchased from BDH chemicals.

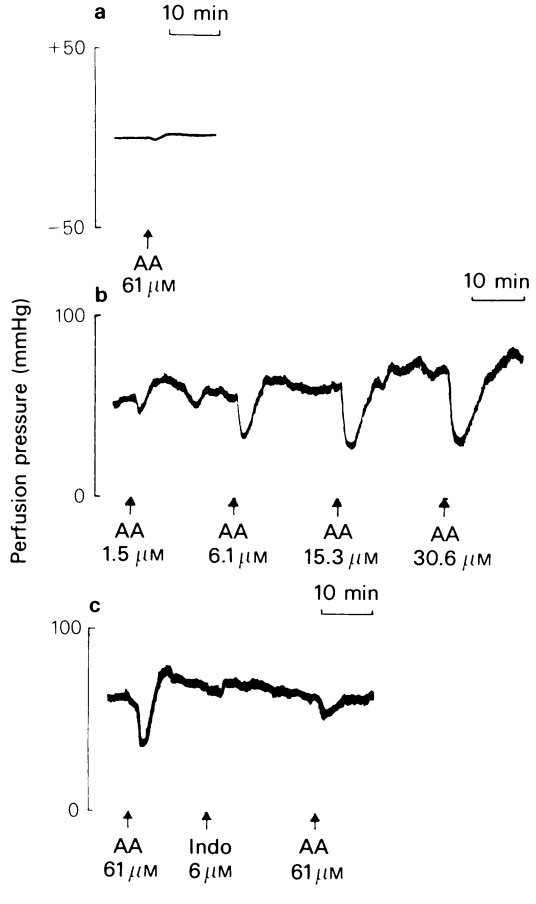

Figure 1 Perfusion pressure recordings from three representative experiments showing (a) the effects of arichidonic acid (AA) on perfusion pressure in normal lung, (b) the relaxation induced by AA in the histamine constricted lung, and (c) the inhibition of AA-induced relaxation by indomethacin (Indo)

Results

An injection of 200 μg of AA (representing an estimated lung concentration of approximately 61 μM) produced little or no modification of the airway perfusion pressure in non-constricted lungs ($n = 4$). However, in lungs where the perfusion pressure was elevated (56–77 mmHg) by histamine (10–40 μM), AA produced a decrease in perfusion pressure representing bronchodilatation (Figure 1). This response to AA was dose-related, although 100% relaxation was never achieved (Figure 2).

Two injections of AA, 30 min apart, resulted in similar degrees of relaxation. In sixteen lungs, AA at 61 μM produced a 25 ± 3 mmHg drop in perfusion pressure (38%) and the second dose produced a 20 ± 2 mmHg decrease in pressure (33%), which was not significantly different from the first. The magnitude of the relaxation did not depend on the absolute perfusion pressure. Regression analysis between these two variables yielded a correlation coefficient of 0.26 which was not significant. An overshoot of perfusion pressure occurred in the lungs on recovery from the relaxation. However, this constriction was not reproducible to successive injections of AA; 13 ± 3 mmHg constriction (26%) following the first administration and 2 ± 2 mmHg (4%) after the second, which was significantly different ($P < 0.005$, $n = 16$).

In an attempt to identify the mechanism underlying the biphasic response to AA, the effects of PGE_1 and PGE_2 on histamine-constricted airways were evaluated. Both PGE_1 and PGE_2 produced a bronchorelaxation ($ED_{50} = 95$ nM and 1,260 nM respectively) which was rapid in onset and in recovery. Furthermore, the relaxant phase was reproducible with PGE_2 whereas the overshoot phenomenon was not.

Where the lungs were perfused with NSAIDs an inhibition of AA-induced relaxation was observed (Figure 3). This effect was in the main dose-related for all six of the NSAIDs tested, with a maximal effect of almost 100% inhibition achieved with indomethacin and phenylbutazone. However, this dose-relationship was weaker in the case of aspirin and phenidone, the limiting factors being solubility in the case of aspirin and intrinsic relaxant activity in the case of phenidone. Both properties were marked at 1,110 μM and 310 μM respectively. Furthermore,

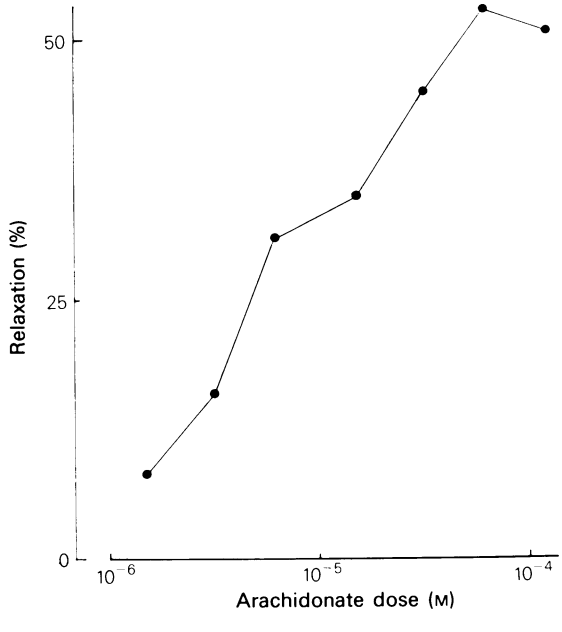

Figure 2 Relaxation of histamine-constricted lung by arachidonic acid (AA). All points are significantly different from control ($P < 0.05$ by Student's 2-tail paired t test); $n = 7$ except for 61 and 123 μM, where $n = 2$.

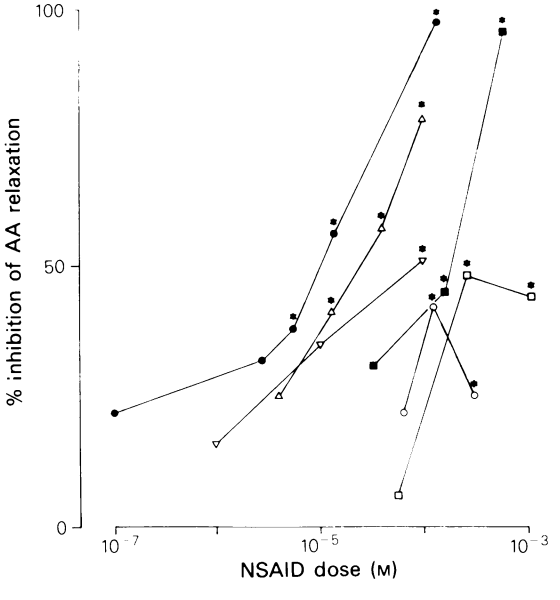

Figure 3 Inhibition of arachidonic acid (AA)-induced relaxation by compounds inhibiting cyclo-oxygenase and lipoxygenase, i.e. NSAIDs, (●) indomethacin, (△) BW755C, (▽) benoxaprofen, (■) phenylbutazone, (○) phenidone and (□) aspirin. All compounds were added to the perfusing fluid, 10 min after the first AA dose and 20 min before the second dose. Significance levels were determined by a 1-tail paired t test $^*P < 0.05$; $n =$ at least 4 for each dose level.

Table 1 Augmentation of histamine-elevated baseline by NSAIDs

Drug	Concentration (μM)	Mean $P_L \pm$ s.e.mean (mmHg) Pre-drug	Post-drug	n	% augmentation	P
Indomethacin	5.6	28.1± 2.3	38.4± 4.2	20	37	<0.001
Aspirin	555	37.8± 3.4	44.4± 3.9	12	17	<0.005
Phenylbutazone	65	38.5±10.7	60.5±17	4	57	<0.01
BW755C	10	43.5± 3.9	47.0± 5.9	4	8	0.8

P_L = intraluminal perfusion pressure; P = significance level derived by Student's 2-tail paired t test.

phenidone was found to inhibit both histamine and barium chloride-induced constrictions in the guinea-pig ileum in a dose-dependent manner.

Perfusion of histamine-constricted lungs with NSAIDs produced significant augmentation of baseline pressure in the cases of indomethacin, phenylbutazone and aspirin, but not for BW755C (Table 1).

Discussion

Our study has demonstrated that perfused intrapulmonary airways under normal tone show little or no response to exogenous AA even at high concentrations. This agrees well with the effects on guinea-pig isolated tracheal chains observed by Lambley & Smith (1975) but conflicts with the data of Mitchell & Denborough (1980). These workers demonstrated a mixture of constrictor and relaxant properties of the trachea, in response to AA, which was dose and tone-dependent.

The addition of exogenous histamine to the perfusion fluid led to an elevation of perfusion pressure indicating a decrease in the calibre of the bronchial tree. Under these conditions AA produced a reproducible and dose-related relaxation of the intrapulmonary airways. In a large proportion of the lungs (75%) the bronchodilator activity was followed by a constrictor/overshoot effect, which was not reproducible. Furthermore, we found that the relaxant response to AA was closely paralleled by that of exogenous PGE_2 suggesting that a PGE_2-like substance may be a major metabolite of AA in our system, a view which is supported by the findings of Hamberg & Samuelsson (1974).

Gryglewski *et al.* (1976) suggest that a differential metabolism of AA exists in constricted lung tissue; parenchymal strips release bronchoconstrictor thromboxanes and tracheal tissue preparations release bronchodilator prostaglandins of the E series. The behaviour of parenchymal strips is believed to reflect accurately that of the peripheral airways (Lulich *et al.*, 1976), although Eyre & Mirbahar (1981) suggest that the reactivity of this tissue is a function of airway, vascular and non-muscular contractile elements. Therefore data indicating that AA produces consistent constriction of parenchymal strips (Mitchell & Denborough, 1980) may not be of any significant relevance to airway smooth muscle. This view is further supported by the observation that AA is a potent constrictor of pulmonary blood vessels (Wicks *et al.*, 1976) and that the gross pharmacological characteristics of parenchymal strips depend on the relative amounts of vascular and airway smooth muscle present (Goldie *et al.*, 1982).

Our measurements of perfusion pressure reflect the mechanical events of the deformation of airways from the main stem bronchus to terminal units, and the results with AA suggest a predominant relaxant activity. We could find no evidence of airway constrictor behaviour unless it was limiting the degree of relaxation obtained. Indeed this may reflect our inability to induce a 100% relaxation of airway smooth muscle with AA. However, in terms of gross intrapulmonary airway response, bronchorelaxation was the fundamental feature.

Evidence that the relaxant effect of AA was indirect, via metabolism by cyclo-oxygenase, was obtained from the observation that the response was inhibited by all the NSAIDs tested in a dose-related manner, except where solubility (i.e. aspirin) and intrinsic smooth muscle relaxant properties (i.e. phenidone) were limiting. Furthermore, the potency level of each compound as an inhibitor of AA responses paralleled its ability to inhibit cyclo-oxygenase in microsomal preparations (Flower, 1974; Willoughby *et al.*, 1981).

The relaxant effect of AA in the whole lung was not converted into a constrictor response following perfusion with indomethacin, which conflicts with the results of Lambley & Smith (1975) and Mitchell & Denborough (1980), using tracheal preparations. However, we found that indomethacin, phenylbutazone and aspirin all produced a significant increase of the histamine-elevated perfusion pressure in their own right. In addition, their rank order of potency as potentiators of constriction and as cyclo-

oxygenase inhibitors was similar. These data agree with observations that agonist-induced bronchoconstriction can be potentiated with NSAIDs (Orehek *et al.*, 1973).

Augmentation of constriction did not occur when lungs were treated with BW755C. This compound inhibits both cyclo-oxygenase and lipoxygenase in polymorphonuclear leukocytes (Higgs *et al.*, 1979) and in lung tissue fragments (Armour *et al.*, 1981). This suggests that while some of the potentiation of bronchoconstriction may be due to the inhibition of relaxant prostaglandin synthesis, part of the effect may be due to enhanced metabolism of AA via the lipoxygenase pathway (Adcock & Garland, 1980; Mitchell, 1982). The primary metabolites from this pathway are the monoeicosatetraenoic acids (HETEs) (Hamberg *et al.*, 1980). Of these, 5- and 15-HETE have been shown to constrict airway smooth muscle with a potency comparable to histamine, as well as potentiating the effects of histamine (Copas *et al.*, 1982).

In conclusion, arachidonic acid relaxes constricted lungs, a response which is inhibited by the NSAIDs. Furthermore, several NSAIDs augment constriction in their own right, although BW755C fails to do so. It is conceivable that both cyclo-oxygenase and lipoxygenase products are associated with the maintenance of intrapulmonary airway tone.

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