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Effects of REV 5901, a 5-lipoxygenase inhibitor and leukotriene antagonist, on pulmonary responses to platelet activating factor in the guinea-pig

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1 The effects of REV 5901 on the platelet activating factor (Paf)-induced (a) bronchoconstriction, (b) contraction of lung parenchymal strips and (c) increased airways responsiveness to histamine, were assessed in the guinea-pig. REV 5901 is a 5-lipoxygenase inhibitor and competitive peptidoleukotriene antagonist which does not inhibit multiple forms of phosphodiesterase.

2 In urethane/pentobarbitone anaesthetized animals, REV 5901 (10 or 30 mg kg^{-1}, i.v.) substantially inhibited the bronchoconstriction, measured as changes in airways resistance (R_L) and dynamic lung compliance (C_{dyn}), induced by leukotriene D_4 (2.5 μg kg^{-1}, i.v.) but did not attenuate that induced by Paf (50 ng kg^{-1}, i.v.).

3 Paf contracted the lung parenchymal strip in a concentration-dependent manner. REV 5901 (25 μM) neither altered the magnitude of the contractions nor the tissue sensitivity to Paf. The sustained contraction induced by Paf was not affected when REV 5901 was added after the response had reached a plateau.

4 Contractions of parenchymal strips to Paf (50 nM) were prevented by pretreatment with the competitive Paf antagonists, SRI 63441 and WEB 2086. Also WEB 2086, but not SRI 63441, reversed established Paf-induced contractions and relaxed parenchymal strips from intrinsic tone in the absence of Paf.

5 Paf (20 ng kg^{-1}, i.v.) caused an acute increase in airways responsiveness to histamine (4–12 μg kg^{-1}, i.v.) which was attenuated by REV 5901 at 10 mg kg^{-1}, i.v. and abolished by 30 mg kg^{-1}, i.v.

6 These data suggest that leukotrienes do not participate in Paf-induced bronchoconstriction or contraction of the lung parenchymal strip, but may play a role in the increased responsiveness of the airways to histamine observed after Paf challenge in the guinea-pig.

Introduction

Platelet activating factor (Paf) represents a family of closely related ether-linked phospholipid mediators of general structure 1-O-aklyl[long chain]-2-acetyl-*sn*-glyceryl-3-phosphorylcholine with pro-inflammatory and bronchoconstrictor properties consistent with a role in asthma (Barnes & Chung, 1987). Although Paf is a potent bronchoconstrictor in animals (Vargaftig *et al.*, 1980; Bonnet *et al.*, 1983; Lewis *et al.*, 1984; Chung *et al.*, 1986) and man (Cuss *et al.*, 1986) it has no direct contractile activity on isolated tracheal or bronchial smooth muscle (Cerrina *et al.*, 1983), suggesting an indirect action through the release of secondary mediators. In addition, Paf causes increased airways responsiveness to diverse spasmogens in experimental animals (Mazzoni *et al.*, 1985; Chung *et al.*, 1986) and man (Cuss *et al.*, 1986) *in vivo*.

A close relationship exists between Paf formation and eicosanoid synthesis. Arachidonic acid is the predominant fatty acid esterified in the Paf precursor, 1-alkyl-2-acyl-*sn*-glycerylphosphorylcholine, and is cleaved from this molecule by phospholipase A_2 during the first step of Paf biosynthesis (Sugiura *et al.*, 1983; Chilton *et al.*, 1984). It may then enter the cyclo-oxygenase and/or lipoxygenase pathways and be metabolized to prostaglandins and thromboxane or leukotrienes. Paf stimulates the release of the potent bronchoconstrictors thromboxane (Lefort *et*

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al., 1984) and leukotrienes (Voelkel *et al.*, 1982; Lefer *et al.*, 1984) from lung tissue and it has been suggested that Paf-induced bronchoconstriction in the guinea-pig *in vivo* is dependent on the formation of leukotrienes (Bonnet *et al.*, 1983).

REV 5901, an arylmethylphenyl ether derivative (α-pentyl-3-(2-quinolinylmethoxy)-benzene methanol), is a selective inhibitor of 5-lipoxygenase and a competitive specific peptidoleukotriene antagonist. The specificity of REV 5901, including the lack of inhibition of multiple forms of phosphodiesterase (Van Inwegen *et al.*, 1987), suggests this compound may be a useful tool for investigation of the role of leukotrienes in biological systems. In the present study the effects of REV 5901 on Paf-induced pulmonary responses in the guinea-pig have been examined.

Methods

Measurement of airways resistance (R_L) and dynamic lung compliance (C_{dyn})

Dunkin-Hartley guinea-pigs of either sex weighing 360–420 g were used in all experiments. Changes in R_L and C_{dyn} were measured as indices of constriction of central and peripheral airways, respectively, in animals anaesthetized with a mixture of urethane/pentobarbitone (25%/0.3% w/v in distilled water, 4–5 ml kg^{-1}, i.p.) by use of a previously described whole body plethysmographic technique (Stewart *et al.*, 1984). Animals were pretreated with pancuronium (1.0 mg kg^{-1}, i.v.) and mechanically ventilated with air by a Palmer pump at 0.7 ml 100 g^{-1} body weight delivered at 60 strokes min^{-1}. Transpulmonary pressure, airflow rate and tidal volume were measured by a Pye differential pressure transducer, Statham pneumotachograph and Grass Model 7P10B integrator, respectively. Phasic blood pressure was determined via a heparinized cannula in the left carotid artery connected to a Druck PDCR 75 pressure transducer. Responses were displayed on a Grass model 79D polygraph. Animals were allowed to stabilize for 30 min after surgical preparation before experiments were started.

The effect of REV 5901 on Paf-induced bronchoconstriction was determined by pretreating animals with this compound or its vehicle 10 min before challenge with a single bolus of Paf (50 ng kg^{-1}, i.v.), a dose selected to produce marked but sub-lethal bronchoconstriction. In separate experiments, animals were challenged with a single bolus of leukotriene D_4 (LTD_4, 2.5 μg kg^{-1}, i.v.) to ensure that REV 5901 was active in the system. This dose of LTD_4 was selected from pilot experiments to induce a bronchoconstriction of comparable magnitude to that caused by 50 ng kg^{-1} Paf. In addition, REV 5901 (30 mg kg^{-1}, i.v.) was found to prevent LTD_4-induced (0.1–1.0 μg kg^{-1}, i.v.) bronchoconstriction throughout a 100 min test period.

To determine the effect of REV 5901 on Paf-induced increased responsiveness to histamine, animals were treated with this compound or its vehicle 10 min before reproducible control responses to histamine were obtained (4–12 μg kg^{-1}, i.v.) causing an increase in R_L of approximately 100% above basal value and an approximate 50% reduction in C_{dyn} below basal value. Animals were then challenged with a single bolus dose of Paf (20 ng kg^{-1}, i.v.). Four responses to the control dose of histamine were then obtained. All responses were elicited at 7 min intervals. The dose of Paf was chosen to produce reversible changes in airways parameters with minimal changes in cardiovascular function (Anderson & Fennessy, 1987). The lungs were reinflated to 3 tidal volumes 120 s after Paf or histamine to maintain a constant volume history within and between experiments. Histamine, Paf and LTD_4 were administered as bolus doses in constant volumes of 0.15 ml. Other drugs were infused over 2–3 min in a volume not exceeding 0.2 ml. Cannulae were flushed with 0.15 ml of saline.

In vitro *experiments*

Animals were killed by a blow to the head and paired strips of lung parenchyma of approximate dimensions 3 × 3 × 30 mm were trimmed from the marginal edges of the left and right caudal lobes. Tissues were suspended at 0.5 g isotonic tension in 25 ml organ baths at 37°C in gassed (95% O_2 plus 5% CO_2) Krebs solution (pH 7.4) of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgCl_2$ 0.5, Na_2HPO_4 1.0 and glucose 11.1. Tissues were washed at 10 min intervals over 1 h during an equilibration period and were then challenged with carbachol 1 mM to induce a standardizing contraction which was defined as 100%. After a further 1 h wash out, during which time the tissues returned to pre-challenge length, drug or vehicle was added to the bath 30 min before challenge with Paf. Contractions to Paf were observed over 2 h. In some experiments drugs were added to the bath after Paf responses had reached a plateau. Since parenchymal strips show tachyphylaxis to Paf, a fresh lung strip was used for each concentration of Paf.

The pD_2 values ($-\log_{10}$ molar concentration producing half maximal response) for Paf-induced contraction were calculated by linear regression of pooled data. The IC_{50} values for the antagonists (defined as the molar concentration of drug reducing the contraction induced by 50 nM Paf to 50% of the

response in paired control tissues) were determined by regression of pooled data. pA_2 values for antagonists were not determined because of the very large number of animals required to obtain an accurate estimate. In experiments where drugs were added after the peak of Paf-induced contraction, the $-\log EC_{50}$ value for relaxation of the tissues (defined as $-\log_{10}$ molar concentration producing half of the maximal net relaxation) was determined by regression of the linear portion of individual concentration-response curves. Drugs were added in a volume of 60 μl or less. Responses were measured with Ugo Basile isotonic transducers (Model No. 7006) and displayed on modified Rikadenki chart recorders (Model No. R02).

Materials

The drugs and suppliers were as follows: carbachol, histamine diphosphate, synthetic Paf, bovine serum albumin (BSA) grade V (Sigma); urethane (B.D.H. chemicals); pancuronium bromide (Organon); sodium pentobarbitone (Abbot Laboratories); synthetic LTD_4 (Merck Frosst, Canada); REV 5901 (α-pentyl-3-(2-quinolinylmethoxy)-benzene methanol) (Rorer group); SRI 63441 (*cis*-(±)-1-[2-[hydroxy[tetrahydro - 5 - [[octadecylcarbonyl]oxy]-methyl]furan - 2 - yl]methoxyphosphinyloxy]ethyl]-quinolium hydroxychloride (Sandoz); WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6-*H*-thieno(3, 2-)(1, 2, 4)-triazolo-(4,3,−α)(1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone (Boehringer Ingelheim).

All other chemicals were of analytical quality or better. Paf was reconstituted from evaporated chloroform stock solution in saline containing 0.25% BSA as carrier in siliconized glassware. Synthetic LTD_4 was diluted in saline from ultrapure H_2O stocks kept at −40°C immediately before use. REV 5901 was dissolved in saline:ethanol:polyethyleneglycol 400 (1:2:1, v/v) for use *in vivo* (volume < 0.05 ml 100 g^{-1}) or in ethanol for *in vitro* experiments. SRI 63441 and WEB 2086 were dissolved in distilled water and diluted in Krebs.

Statistics

Data are presented as the mean response ± standard error of the mean (s.e. mean) for n observations. *In vivo* experiments required comparison of several treatment groups to a single control group and were analysed by one-way analysis of variance (ANOVA) followed, where appropriate, by Dunnett's test comparing responses at corresponding time points. *In vitro* results were analysed by paired Student's t test. Results were considered to be significantly different for $P < 0.05$.

Results

Effect of REV 5901 on Paf-induced bronchoconstriction

The effects of REV 5901 on Paf- and LTD_4-induced bronchoconstriction are shown in Table 1. Paf (50 ng kg^{-1}, i.v.) caused a significant ($P < 0.01$), marked and persistent bronchoconstriction comprising an increase in R_L of 0.49 ± 0.28 cm H_2O ml^{-1} s^{-1} and a fall in C_{dyn} of 0.10 ± 0.01 ml cm H_2O^{-1}, which was accompanied by a sustained fall in blood pressure. LTD_4 (2.5 μg kg^{-1}, i.v.) induced a significant ($P < 0.01$) bronchoconstriction of comparable magnitude (increase in R_L: 0.52 ± 0.14 cm H_2O ml^{-1} s^{-1}; decrease in C_{dyn}: 0.14 ± 0.07 ml cm H_2O^{-1}). REV 5901 at 10 mg kg^{-1}, i.v., significantly reduced ($P < 0.05$) and at 30 mg kg^{-1} almost abolished ($P < 0.01$) the responses to LTD_4. However, REV 5901 had no significant effect on Paf-induced bronchoconstriction at either dose. In addition REV 5901 did not alter the rate of onset of Paf-induced bronchoconstriction or the duration of the response.

Paf-induced increased airway responsiveness to histamine

The effect of REV 5901 pretreatment on the increased airway responsiveness induced by Paf is presented in Figure 1a, b and c. Paf (20 ng kg^{-1}, i.v.) caused a transient increase in the magnitude of responses to histamine (4–12 μg kg^{-1}, i.v.) in both central and peripheral airways, as indicated by the changes in R_L and C_{dyn}, respectively, (Figure 1a). This effect was substantially attenuated by REV 5901 at 10 mg kg^{-1} (Figure 1b) and was abolished by the 30 mg kg^{-1} dose (Figure 1c). At the higher dose of REV 5901 the responses to histamine elicited after Paf challenge were slightly but not significantly attenuated; REV 5901 *per se* was found to have no direct effect on airways sensitivity to histamine in doses as high as 80 mg kg^{-1}, i.v. (data not shown).

Lung strips in vitro

Paf contracted the guinea-pig lung strip in a concentration-dependent manner (Figure 2). Responses of the lung strip to Paf were not significantly altered by REV 5901 25 μM: pD_2 values (95% confidence limits) were for the ethanol vehicle control, 7.40 (6.92–7.92) and for REV 5901, 7.38 (6.89–7.87). In addition, REV 5901 did not alter either the rate of onset or duration of contraction. When added to the organ bath approximately 90 min after Paf challenge (1 μM), when the response had reached a stable plateau, REV 5901 produced a

Table 1 Effect of REV 5901 pretreatment on leukotriene D_4 (LTD_4) and platelet-activating factor (Paf)-induced bronchoconstriction *in vivo*

			Airways response	
			Increase in R_L	*Decrease in* C_{dyn}
Pretreatment	*Challenge*	n	($cm\,H_2O\,ml^{-1}\,s^{-1}$)	($ml\,cm\,H_2O^{-1}$)
Vehicle	Saline	4	0.022 ± 0.020	0.010 ± 0.022
Vehicle	LTD_4	5	0.526 ± 0.147^{a}	0.149 ± 0.072^{a}
REV 10	LTD_4	6	0.219 ± 0.103^{ab}	0.142 ± 0.080^{ab}
REV 30	LTD_4	6	0.054 ± 0.093^{b}	0.034 ± 0.012^{b}
Vehicle	BSA[1]	4	0.010 ± 0.022	0.003 ± 0.003
Vehicle	Paf	8	0.491 ± 0.283^{c}	0.096 ± 0.021^{c}
REV 10	Paf	8	0.395 ± 0.139^{c}	0.072 ± 0.011^{c}
REV 30	Paf	6	0.860 ± 0.352^{c}	0.091 ± 0.016^{c}

Animals were surgically prepared to determine R_L and C_{dyn}. Ten minutes prior to challenge with a single bolus dose of LTD_4 ($2.5\,\mu g\,kg^{-1}$, i.v.) or Paf ($50\,ng\,kg^{-1}$, i.v.), animals were pretreated with vehicle (saline : ethanol : polyethyleneglycol 400; 1:2:1 v/v; $<0.05\,ml\,100\,g^{-1}$, i.v. infusion) or REV 5901 (10.0 or $30.0\,mg\,kg^{-1}$, i.v. infusion). Data, presented as the mean ± s.e. mean of the maximum changes in R_L or C_{dyn} from pre-challenge values, were analysed by ANOVA and Dunnett's test for comparison of several treatment groups to a single vehicle control group.

[1] 0.25% BSA in saline (carrier solution for Paf).

[a] $P < 0.05$ compared to vehicle pretreated, saline challenged control group.

[b] $P < 0.05$ compared to vehicle pretreated, LTD_4 challenged group.

[c] $P < 0.05$ compared to vehicle pretreated, BSA challenged control group.

slight relaxation which was not concentration-dependent but which was equivalent in magnitude to the relaxation caused by ethanol vehicle (Figure 3). In contrast, the competitive Paf antagonist, WEB 2086, concentration-dependently reversed the contraction induced by 1.0 μM Paf ($-\log EC_{50}$: 5.97) and relaxed tissues beyond their initial basal length. However, another selective Paf antagonist, SRI 63441, did not reverse the Paf-induced contraction (Figure 3). In separate experiments WEB 2086, but not SRI 63441, directly relaxed lung parenchymal strips from intrinsic tone with a comparable $-\log EC_{50}$ value of 5.82 (Figure 3). Both WEB 2086 and SRI 63441 antagonized contractions of the parenchymal strip when added to the bath prior to Paf (50 nM) challenge: their IC_{50} values were $1.6 \pm 0.4\,\mu M$ and $8.5 \pm 1.0\,\mu M$, respectively.

Discussion

The principal findings of this study indicate that REV 5901, a selective antagonist of peptidoleukotrienes and an inhibitor of 5-lipoxygenase, neither altered Paf-induced bronchoconstriction *in vivo* nor contraction of the lung parenchymal strip *in vitro* in the guinea-pig. In contrast, REV 5901 prevented, in a dose-dependent manner, the Paf-induced increase in airways responsiveness to histamine *in vivo*.

Paf induces the formation of leukotrienes *in vitro* in a number of cells and tissues including human eosinophils (Bruynzeel *et al.*, 1986), rat perfused lung (Voelkel *et al.*, 1982) and chopped lung tissue of the cat (Lefer *et al.*, 1984). However, the involvement of leukotrienes in Paf-induced bronchoconstriction *in vivo* is controversial. Bonnet *et al.* (1983) examined the actions of a number of drugs thought to interfere with leukotriene synthesis or action and concluded that lipoxygenase metabolites mediate Paf-induced bronchoconstriction. In contrast, 5,8,11,14-eicosatetraynoic acid (ETYA), an inhibitor of both cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism, did not prevent Paf-induced bronchoconstriction (Vargaftig *et al.*, 1980). Lewis *et al.* (1984) examined the effects of FPL 55712, a leukotriene antagonist, nordihydroguaretic acid (NDGA), an anti-oxidant which inhibits lipoxygenases, and two dual inhibitors of cyclo-oxygenase and lipoxygenase, phenidone and BW 755C, on Paf-induced bronchoconstriction *in vivo*. In the latter study, FPL 55712 inhibited Paf-induced bronchoconstriction but only when administered at a non-specific dose ($10\,mg\,kg^{-1}$, i.v.) whereas aerosolized FPL 55712 (1% solution) was without effect. While NDGA significantly inhibited bronchoconstriction, the specificity of this compound *in vivo* is questionable. Inhibition of cyclo-oxygenase product formation by BW 755C may have contributed to its effect. In the present study REV 5901 had no effect on Paf-induced

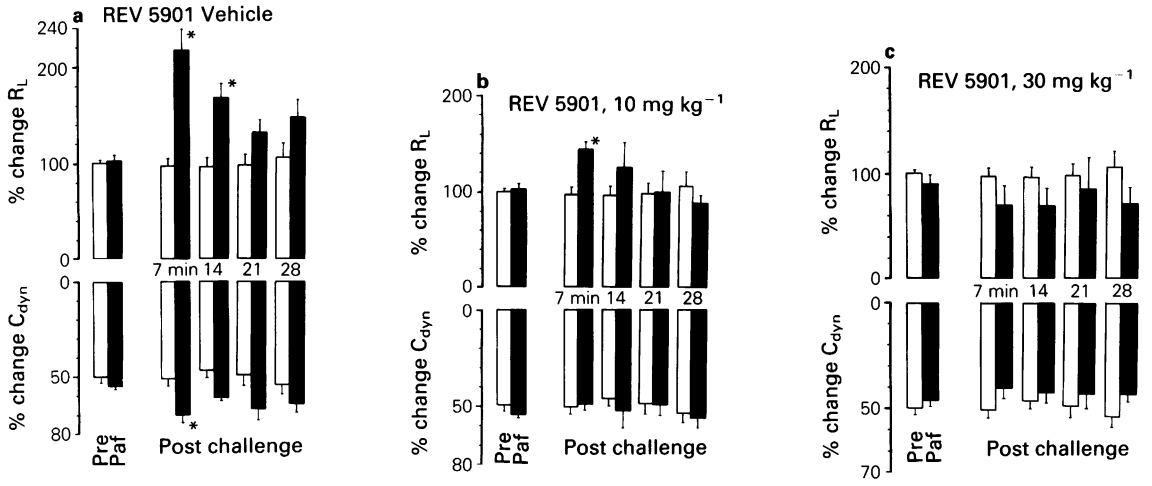

Figure 1 Effects of REV 5901 on the Paf-induced increase in airways responsiveness to histamine. Animals, surgically prepared for whole body plethysmography, were pretreated with vehicle (a), REV 5901 10 mg kg^{-1}, i.v. (b) or REV 5901 30 mg kg^{-1}, i.v. (c) 10 min before histamine at a dose (4–12 μg kg^{-1}, i.v.) that reproducibly increased R_L by 100% above resting levels. Animals were then challenged with a single i.v. bolus of BSA carrier solution or Paf (20 ng kg^{-1}, i.v.). Responses to histamine, at the prechallenge dose, were then elicited. All responses were obtained at 7 min intervals. Open columns denote responses in the control group before and after BSA challenge. Closed columns denote responses in Paf-challenged groups. Error bars denote s.e. mean. * indicates a significant difference, $P < 0.05$, from BSA challenged, control histamine responses at the corresponding time point.

bronchoconstriction when administered in doses that prevented LTD_4-induced bronchoconstriction of a comparable magnitude to Paf. This suggests that leukotrienes do not contribute substantially to this response. The failure of AA 861, a selective 5-lipoxygenase inhibitor, to alter Paf-induced bronchoconstriction in the guinea-pig (Barton *et al.*, 1987) further supports the lack of involvement of leukotrienes.

Similarly, the involvement of eicosanoids in Paf-induced contraction of the guinea-pig lung parenchymal strip is equivocal. Stimler & O'Flaherty (1983) found no effect using the cyclo-oxygenase inhibitors, aspirin, indomethacin or sulphinpyrazone, the lipoxygenase inhibitors, NDGA or ETYA, and the leukotriene antagonist FPL 55712. In contrast, Detsouli *et al.* (1985) observed that the inhibitors of leukotriene synthesis, NDGA, ETYA, BW 755C or diethylcarbamazine, and a phospholipase A_2 inhibitor, *para*-bromophenacylbromide, reduced the magnitude of Paf-induced contractions, but only at non-selective concentrations. In the same study FPL 55712 was without effect. In addition, Touvay *et al.* (1986) found NDGA, aspirin and the thromboxane synthetase inhibitors, imidazole and OKY 046, reduced Paf-induced contraction, but FPL 55712 had no effect. The general lack of effect of inhibitors of either leukotriene synthesis or action on responses to Paf in the lung strip is supported by the present findings. REV 5901 was without effect on the magnitude, duration or maintenance of Paf responses when applied in a concentration greater than that reported either to inhibit responses to exogenous peptidoleukotrienes (Van Inwegan *et al.*, 1987) or to prevent antigen-induced leukotriene synthesis in sensitized guinea-pig lung tissue (Tennant *et al.*, 1987).

The competitive Paf antagonists, WEB 2086 and SRI 63441, reduced the magnitude of Paf-induced contractions in a concentration-dependent manner when applied before Paf challenge, suggesting that Paf initiates contraction through a receptor-mediated mechanism. Only WEB 2086 reversed the sustained contraction when applied after the response to Paf had reached plateau. However, as WEB 2086 also relaxed lung strips from intrinsic tone, functional antagonism may be responsible for this effect rather than Paf-receptor antagonism. It is possible that the relaxant effect of WEB 2086, observed in the present study, may contribute to the attenuation of anaphylactic bronchoconstriction in actively or passively sensitized guinea-pigs *in vivo* and *in vitro* (Casals-Stenzel, 1987; Pretolani *et al.*, 1987). Alternatively, since there is some evidence for the existence of Paf receptor subtypes in isolated cells (Lambrecht & Parnham, 1986) and in rat isolated lung (Voelkel *et al.*, 1986) the possibility that Paf may initiate and sustain contraction through separate receptors cannot be excluded. The failure of SRI 63441 to reverse the sustained phase of Paf-induced contraction requires further investigation.

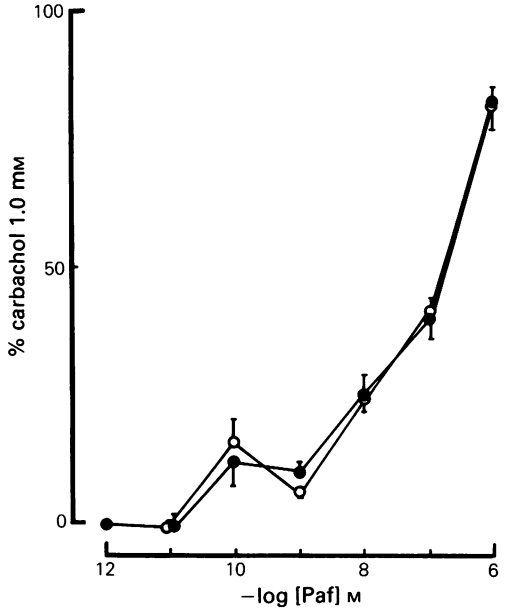

Figure 2 Concentration-response curve to Paf in lung strips in the presence of REV 5901 25 μM. Paired lung strips were equilibrated with REV 5901 (●) or ethanol vehicle (○) for 30 min before challenge with a single concentration of Paf. Responses represent the maximum contraction in tissues observed over 2 h and are expressed as a percentage of the response to carbachol 1.0 mM which was defined as 100%. Separate lung strips were used for each response and data are presented as the means with s.e. mean shown by vertical bars. $n = 6$–10 per Paf concentration. Analysis by paired t test at each concentration of Paf showed no significant differences between control and treated groups.

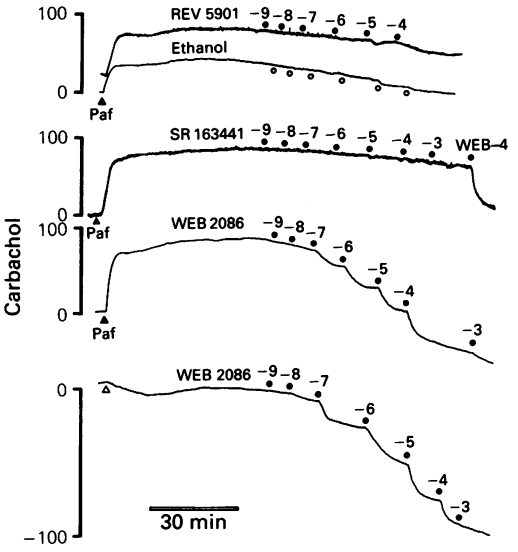

Figure 3 Effects of drugs on the sustained phase of Paf-induced contraction of lung parenchymal strips. Tissues contracted to Paf (▲, 1.0 μM) for 90 min, were exposed to cumulatively increasing concentrations (10^{-9}–10^{-3} M) of REV 5901, SRI 63441, WEB 2086 or ethanol vehicle (0.1–0.6%, v/v). The bottom panel shows the effect of WEB 2086 on basal tone of tissues exposed only to Paf vehicle (△, 0.25% BSA in saline). (●) denotes addition of drug at the indicated molar concentration and (○) denotes addition of vehicle. Responses are expressed as a percentage of the response to carbachol 1.0 mM in each tissue. Concentrations of WEB 2086 producing half maximal reduction of tone were determined by linear regression. 4–6 experiments were performed for each treatment.

In addition to its potent bronchoconstrictor activity, Paf causes an increase in airways responsiveness to a range of spasmogens in experimental animals (Chung *et al.*, 1986; Mazzoni *et al.*, 1986) and healthy humans (Cuss *et al.*, 1986). The mechanism of this effect is also not clear although eicosanoids may be involved. In the dog, Paf-induced increased responsiveness to acetylcholine is prevented by OKY 046 suggesting the involvement of thromboxane (Chung *et al.*, 1986). However in the guinea-pig, aspirin and indomethacin, which inhibit thromboxane formation at the level of cyclo-oxygenase, enhance Paf-induced increased responsiveness (Mazzoni *et al.*, 1985; Anderson & Fennessy, 1987). It is possible that this enhancement by cyclo-oxygenase inhibitors may be due to shunting of arachidonic acid towards lipoxygenase pathways after Paf challenge. We have previously demonstrated that BW 755C, ETYA and FPL 55712, but not aspirin or indomethacin, prevent Paf-induced increases in airways responsiveness to histamine in guinea-pigs which suggests the involvement of lipoxygenase products (Anderson & Fennessy, 1987).

Exogenous LTD_4 increased airways responsiveness to histamine in the guinea-pig (Stewart *et al.*, 1984; Fennessy *et al.*, 1986) whereas in healthy humans LTD_4 augmented airways responses to methacholine (Kern *et al.*, 1986) and prostaglandin $F_{2\alpha}$ (Barnes *et al.*, 1984a) but not to histamine (Barnes *et al.*, 1984b). The inhibitory effect of REV 5901 in the present study suggests that lipoxygenase metabolites may mediate altered responsiveness after Paf challenge. The possibility that eicosanoids, particularly leukotrienes, may mediate increased airways responsiveness after inhaled Paf in man has not been tested although indomethacin did not prevent Paf-induced bronchoconstriction (Rubin *et al.*, 1987). In a recent trial REV 5901 (1 g, orally) failed to have any effect on inhaled LTD_4-induced bronchoconstriction in human volunteers (Barnes,

1987) so the resolution of this problem must await the availability of further safe effective inhibitors of 5-lipoxygenase or leukotriene antagonists. Nevertheless, the present findings do suggest that 5-lipoxygenase metabolites released subsequently to Paf challenge do not contribute substantially to bronchoconstriction but may mediate an increased airways responsiveness to histamine in the guine-pig.

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