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An *in vivo* model for measuring antigen-induced SRS-A-mediated bronchoconstriction and plasma SRS-A levels in the guinea-pig

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1 Pharmacological modulation of antigen-induced anaphylaxis in actively sensitized guinea-pigs with intravenously administered indomethacin (10 mg/kg), pyrilamine (2.0 mg/kg) and propranolol (0.1 mg/kg) resulted in a delayed onset, slowly developing bronchoconstriction indicative of a slow-reacting substance of anaphylaxis (SRS-A) response.

2 Measurements of pulmonary mechanics on the drug-pretreated animals challenged with ovalbumin demonstrated a more prominent effect on dynamic compliance than resistance. This is consistent with the more potent effects of SRS-A on peripheral rather than central airways.

3 The slowly developing bronchoconstriction obtained after treatment with indomethacin, pyrilamine and propranolol was inhibited by the standard SRS-A antagonist, FPL 55712 and the SRS-A synthesis inhibitors, phenidone, BW 755C and nordihydroguaiaretic acid.

4 Plasma SRS-A levels were determined in guinea-pigs following antigen challenge. The appearance of SRS-A in the plasma preceded the onset of bronchoconstriction and SRS-A levels remained elevated throughout its development. Coincident with the inhibition of bronchoconstriction by the SRS-A synthesis inhibitor, phenidone, was a dose-dependent reduction in plasma SRS-A. The intravenous ED_{50} in each case was 4 mg/kg.

5 This model of antigen-induced SRS-A-mediated bronchoconstriction should prove useful for the *in vivo* evaluation and development of therapeutics which regulate the synthesis of SRS-A.

Introduction

Slow reacting substance of anaphylaxis (SRS-A) is believed to be an important mediator in the pathogenesis of asthma (Piper, 1980; Samuelsson, Hammarstrom, Murphy & Borgeat, 1980). SRS-A is derived from the metabolism of arachidonic acid via a lipoxygenase initiated series of enzymatic reactions and comprises a family of eicosenoid compounds containing a conjugated triene system of double bonds and varying amino acid substitution at the six carbon position. Two of these compounds, leukotriene C (LTC_4; 5(S)-hydroxy, 6(R)-S-glutathionyl-7,9-*trans*,11, 14-*cis*-eicosatetraenoic acid) and leukotriene D (LTD_4; 5(S)-hydroxy,6(R)-S-cysteinyl-glycine-7,9-*trans*,11,14,*cis*-eicosatetraenoic acid) are believed to be the major components of SRS-A (Bach, Brashler, Brooks & Neerken, 1979; Morris, Taylor, Piper, Samhoun & Tippins, 1980). Further metabolism of LTD_4 to leukotriene E (LTE_4; 5(S)-hydroxy,6(R)-S-cysteiny-7,9-*trans*,11,14-*cis*-eicosatetraenoic acid) also a potent bronchoconstrictor (Lewis, Drazen, Austen, Toda, Brion, Marfat & Corey, 1981; Welton, Crowley, Miller & Yaremko, 1981) has been demonstrated to occur in rat peritoneal cells (Parker, Falkenhein & Huber, 1980) and human blood (Parker, Koch, Huber & Falkenhein, 1980).

The development of compounds designed to modulate the pathophysiological effects of the leukotrienes by inhibition of their *de novo* biosynthesis has been hampered by the lack of a suitable *in vivo* model. This study describes an *in vivo* model of antigen-induced changes in ventilatory pressure mediated by the endogenous production of SRS-A, which can distinguish between SRS-A synthesis inhibitors and SRS-A antagonists by the determination of plasma SRS-A levels.

Methods

Male Hartley guinea-pigs (200–300 g) were sensitized with a single intraperitoneal injection of ovalbumin (10 mg) in 1 ml of 0.9% w/v NaCl solution (saline). The animals were used for study on days 28–45, following the ovalbumin injection. Sensitized guinea-pigs were anaesthetized with an intraperitoneal injection of urethane (2 g/kg), and the carotid artery and jugular vein cannulated for meas-

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urement of arterial blood pressure and to facilitate intravenous drug administration. Animals were ventilated with a Harvard small animal respirator set at a stroke volume of 2.5 cc and a rate of 40 breaths per min. Ventilatory pressure (cmH_2O) was measured with a Statham pressure transducer from a side arm off the tracheal cannula.

Following surgical preparation, the animals were pretreated with indomethacin (10 mg/kg, i.v.) 22 min before ovalbumin challenge presumably to shunt arachidonic acid from the cyclo-oxygenase pathway into the lipoxygenase pathway for the formation of leukotrienes, and eliminating the modulating effects of prostaglandins. Spontaneous breathing was arrested with succinylcholine (1.2 mg/kg) 7 mins before antigen challenge. Treatment with succinylcholine resulted in a constant baseline ventilatory pressure of 5–8 cmH_2O. The bronchoconstrictor effects of histamine were abolished with pyrilamine (2.0 mg/kg), administered 6 min before antigen challenge. Since previous studies have demonstrated a potentiating effect of propranolol (0.1 mg/kg) on bronchoconstriction induced with synthetic LTE_4 (Welton *et al.*, 1981), propranolol was administered 5 min before antigen challenge.

Pulmonary mechanics

In addition to the animal preparation described above, transpulmonary pressure was measured by inserting an 18 gauge hypodermic needle through the fifth or sixth intercostal space near the sternum and into the intrapleural space. One side of the differential pressure transducer was attached to the pleural needle, and the other side connected to a side arm off the tracheal cannula. Airflow was measured with a Fleisch pneumotachograph. Electrical integration of the flow signal provided a recording of tidal volume. Pulmonary resistance and dynamic lung compliance were calculated manually from the transpulmonary pressure, airflow and volume, as described by Amdur & Mead (1958), and used to calibrate a Buxco on-line analogue pulmonary computer. After surgically preparing the animals for physiological monitoring, a period of not less than 15 min was allowed for pulmonary functions to stabilize.

Determination of plasma SRS-A

Arterial blood was drawn from the carotid artery of the guinea-pigs into sodium citrate (0.38%) containing indomethacin (10^{-4} M). Blood volume was replaced with isotonic saline. The blood samples were centrifuged at 1200 *g* for 15 min at 4°C. The plasma supernatant was decanted and extracted with 4 volumes of absolute ethanol. After allowing the samples to stand on ice for 30 min, the precipitated protein was removed by centrifugation at 40,000 *g* for 30 min at 4°C. The ethanol supernatant was decanted and evaporated to dryness under nitrogen. Samples were redissolved in distilled water for bioassay.

SRS-A was bioassayed on the guinea-pig ileum incubated in Tyrode solution (composition mM: NaCl 136.7, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2.6H_2O$ 1.1, $NaH_2PO_4.H_2O$ 0.48, $NaHCO_3$ 23.8 and glucose, 11.1) containing 10^{-6} M atropine sulphate and 10^{-6} M pyrilamine maleate and aerated with 95% O_2 and 5% CO_2. The temperature of the assay bath was maintained at 37° with a Tamson circulating water bath. Changes in tension were measured isotonically with an Adaps rotary motion transducer and recorded on a Linear recorder. The tissue was standardized with chemically synthesized LTE_4 (Rosenberger & Neukom, 1980). Plasma extracted SRS-A values are given in equivalents of standard LTE_4 doses.

Materials

The following drugs were used: Indomethacin, propranolol hydrochloride, pyrilamine maleate, ovalbumin grade V, succinylcholine chloride, and urethane (Sigma Chemical Company, St Louis, MO); 1-phenyl-3-pyrazolidone (phenidone, Aldrich Chemical Co., Milwaukee, WI); 3-amino-1 [M(trifluoromethyl)-phenyl]-2-pyrazoline (BW 755C, Burroughs Wellcome, Research Triangle Park, NC); sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy) - 2 - hydroxypropoxy] - 4 - oxo - 8 -propyl-4H-1-benzopyran-2-carboxylate (FPL 55712, Fisons Ltd; Loughborough, Leicester); 2-(*p*-cholorophenyl)α-methyl-5-benzoxazole acetic acid (Benoxaprofen, Eli Lilly, Indianapolis, IN); nordihydroguairetic acid (NDGA) and 5,8,11,14-eicosatetraynoic acid (ETYA, Hoffmann La-Roche, Nutley, NJ). Concentrations are given as the salt of the compound used. All drugs were administered in saline whenever possible or polyethylene glycol: ethanol (50:50) and injected in volumes less than 500 µl. ETYA was dissolved in 0.1 M sodium carbonate.

Statistics

Statistical significance was determined by Student's *t* test with a significance level of $P < 0.05$.

Results

SRS-A mediated bronchoconstriction

Animals pretreated with indomethacin, succinylcholine, pyrilamine and propranolol as described in the Methods section, were challenged intravenously

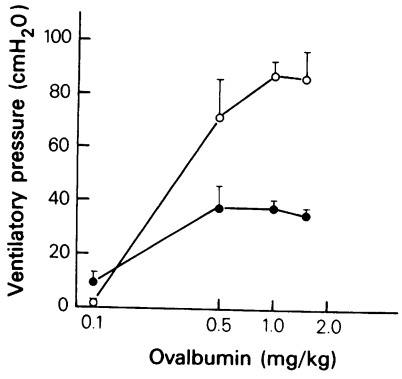

Figure 1 Dose-response curve to ovalbumin. Guinea-pigs were surgically prepared and pretreated with indomethacin (10 mg/kg) propranolol (0.1 mg/kg) and succinylcholine (1.2 mg/kg). A dose-response curve to ovalbumin was then obtained in the presence (●) and absence (○) of pyrilamine (2.0 mg/kg). The response is recorded in ventilatory pressure (cmH_2O) and given as the mean of $n = 9$; vertical lines indicate s.e.mean.

with ovalbumin (0.1–1.5 mg/kg) to determine the optimal dose of antigen to be used in these studies. Only one dose of antigen was tested per animal. The magnitude of the increased ventilatory pressure obtained in these animals was compared to the increases obtained in a similar group of animals which were challenged in the absence of the antihistamine,

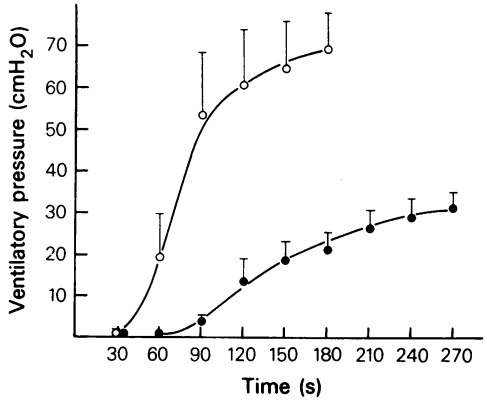

Figure 2 Time course for the development of antigen-induced bronchoconstriction. Guinea-pigs sensitized to ovalbumin were surgically prepared under urethane anaesthesia (2 g/kg) for measuring ventilatory pressure and pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg) and succinylcholine (1.2 mg/kg). Animals were then challenged with ovalbumin (1 mg/kg) in the presence (●) and absence (○) of pyrilamine (2.0 mg/kg). Ventilatory pressure is given in cmH_2O pressure as the mean of $n = 5$; vertical lines indicate s.e.mean.

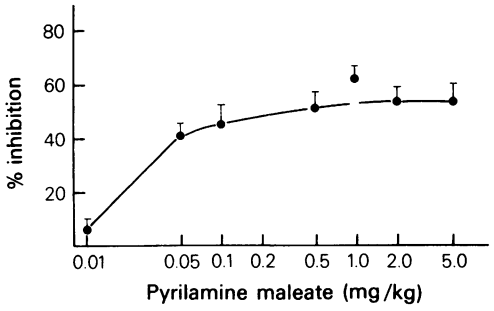

Figure 3 Dose-response curve to pyrilamine. Anaesthesized, surgically prepared animals were pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg) and succinylcholine (1.2 mg/kg) and challenged with ovalbumin (1.0 mg/kg) in the presence of pyrilamine (0.01–5.0 mg/kg). Responses are presented as percentage inhibition of maximum control responses (animals not pretreated with pyrilamine) as the mean of $n = 5$; vertical lines indicate s.e.mean.

pyrilamine. In both groups of animals, increasing doses of antigen produced a dose-dependent increase in ventilatory pressure (Figure 1). As expected, the magnitude of the ventilatory pressures in the absence of pyrilamine was greater than in its presence; however, the relative position of the two dose-response curves to antigen was apparently unaffected by the antihistamine. The maximum response to antigen for both groups of animals was achieved at a dose of 1.0 mg/kg which was used throughout the following experiments.

Animals challenged with 1 mg/kg ovalbumin in the presence of pyrilamine demonstrate a slowly developing increase in ventilatory pressure beginning 60–90 s after antigen challenge, and reaching a maximum response by 300 s (Figure 2). In the absence of pyrilamine, the onset is more rapid, beginning 30–60 s after antigen administration and plateauing by 180 s.

Since the major mediator of anaphylactic bronchoconstriction in the guinea-pig is believed to be histamine (Collier & James, 1967; Ritchie, Sierchio, Capetola & Rosenthale, 1981) it was important to establish that the selected dose of pyrilamine to be used in these studies (2 mg/kg) completely inhibited the changes in ventilatory pressure due to the released histamine. Thus a dose-response curve to pyrilamine (0.01–5.0 mg/kg) was determined (Figure 3). The maximum inhibition obtainable with pyrilamine was 62% and was obtained with a dose as low as 0.5 mg/kg. To substantiate that the remaining response was due to SRS-A, FPL 55712 a standard SRS-A antagonist (Augstein, Farmer, Lee, Sheard & Tattersall, 1973; Chand, 1979) was employed. FPL 55712, administered 30 s before antigen challenge, caused a dose-dependent inhibition of the antihis-

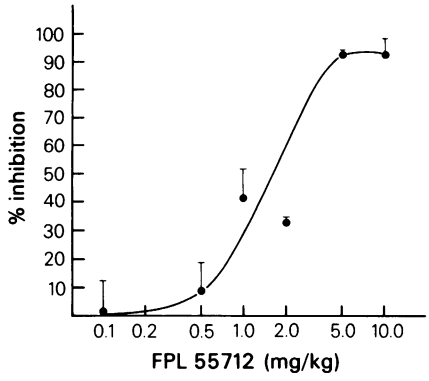

Figure 4 Dose-response curve to FPL 55712. A dose-response curve was determined to FPL 55712 (0.1–10 mg/kg), administered 30 s before antigen challenge. Animals were pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg), pyrilamine (2.0 mg/kg), and succinylcholine (1.2 mg/kg), and challenged with albumin (1.0 mg/kg). Responses are presented as percentage inhibition of control responses as the mean of $n = 4-8$; vertical lines indicate s.e. mean.

tamine resistant increase in ventilatory pressure (Figure 4). Essentially complete inhibition (92 ± 1%) was obtained at a dose of 5 mg/kg with an ED_{50} of approximately 2 mg/kg.

Pulmonary mechanics

Figure 5(a) illustrates the time course of antigen-induced changes in resistance and dynamic compliance in animals pretreated with indomethacin, succinylcholine, pyrilamine, and propranolol. Resistance increased by a maximum of 60% above control with dynamic compliance decreasing 90%. The time course of these changes is consistent with those observed in Figure 2. FPL 55712 administered 30 s before antigen challenge, significantly blocked both resistance and compliance changes.

In contrast (Figure 5b), antigen-induced changes in resistance and dynamic compliance in the absence of the antihistamine, are apparent at 30 s and rapidly plateau by 90 s. Pulmonary resistance demonstrated a peak increase of 100% before levelling at 60% above baseline values. Dynamic compliance dropped sharply to 10% of control values. It is interesting that

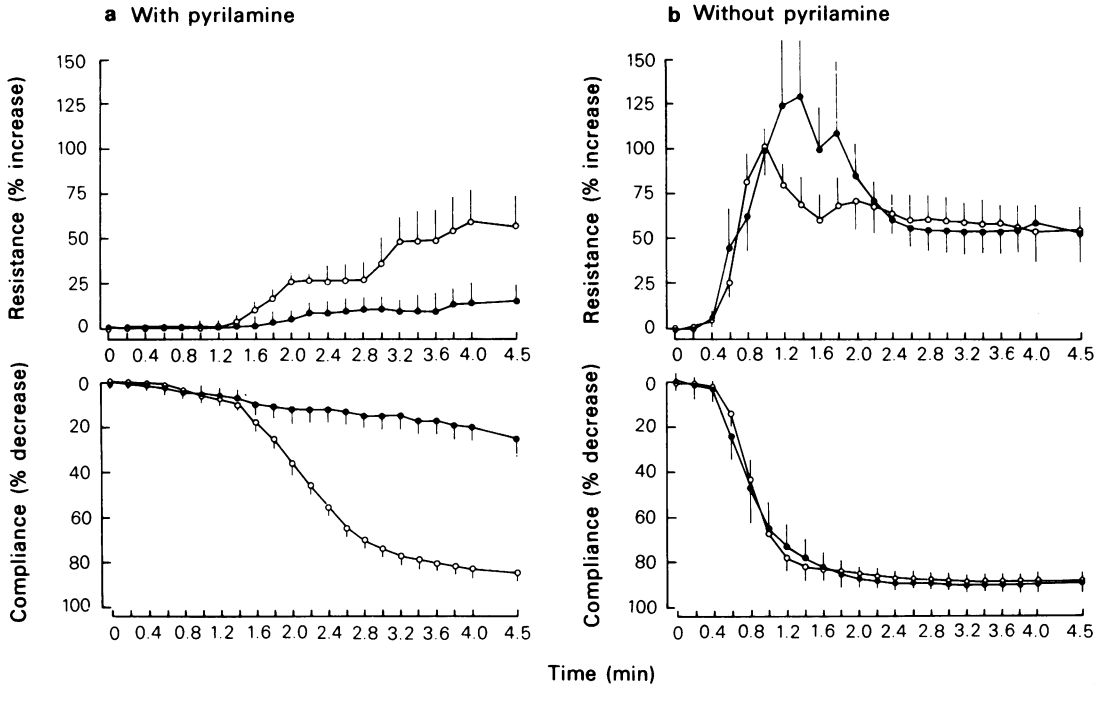

Figure 5 Effects of FPL 55712 on antigen-induced increases in pulmonary resistance and dynamic lung compliance. Animals were surgically prepared and pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg), succinylcholine (1.2 mg/kg) and challenged with ovalbumin (1.0 mg/kg) in the presence (a) or absence (b) of pyrilamine (2.0 mg/kg). The effects of FPL 55712 (10 mg/kg) (●) administered intravenously 30 s before antigen challenge were also tested in the presence of pyrilamine (a) or absence (b). Each point represents the mean of 6 experiments; vertical lines indicate s.e.mean.

Table 1 Effect of SRS-A synthesis inhibitors on antigen-induced increases in ventilatory pressure

Drug (10 mg/kg)	*% inhibition of control response*	n	P
Phenidone	92.3 ± 5.0	5	< 0.001
BW 755C	34.9 ± 5.2	5	< 0.05
NDGA	29.0 ± 6.9	5	< 0.05
ETYA	20.3 ± 5.9	6	NS
Benoxaprofen	17.9 ± 6.9	5	NS

NS = not significant.

in the absence of pyrilamine maleate, FLP 55712 did not signicantly affect either resistance or compliance changes.

SRS-A synthesis inhibitors

Five compounds reported to inhibit SRS-A biosynthesis *in vitro*: phenidone (Blackwell & Flower, 1978; Patterson, Burka & Craig, 1981); BW 755C (Burka & Flower, 1979; Armour, Hughes, Seale & Temple, 1981; Piper & Temple, 1981), NDGA (Armour *et al.*, 1981), ETYA (Hitchcock, 1978; Patterson *et al.*, 1981; Orning & Hammarstrom, 1980) and benoxaprofen (Walker, Boot, Cox & Dawson, 1980) were studied for their effect on SRS-A mediated bronchoconstriction *in vivo* (Table 1). Phenidone, BW 755C and NDGA at a dose of 10 mg/kg all caused a significant reduction in bronchoconstriction when administered intravenously 60 s before antigen challenge. Phenidone was the most effective agent tested, inhibiting bronchoconstriction by 92.3 ± 5.0%. BW755C and NDGA produced 34.9 ± 5.2% and 29.0 ± 6.9% inhibition, respectively. ETYA and benoxaprofen produced no statistically significant inhibition.

Determination of plasma SRS-A levels

The time course for the appearance of SRS-A-like activity in the plasma of animals pretreated with indomethacin, succinylcholine, pyrilamine and propranolol and challenged intravenously with antigen is presented in Figure 6. This time course is compared to the development of bronchoconstriction in the

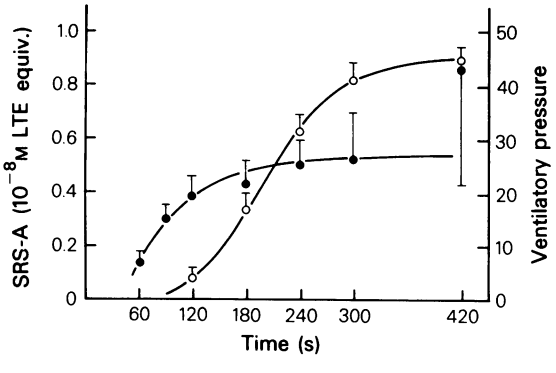

Figure 6 Time course of antigen-induced bronchoconstriction (○) and the appearance of SRS-A (●) in plasma. Guinea-pigs were surgically prepared and pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg), pyrilamine (2.0 mg/kg) and succinylcholine (1.2 mg/kg). The animals were then challenged with ovalbumin (1.0 mg/kg) and 1 ml blood samples were drawn into sodium citrate (0.38%) containing indomethacin (10^{-4} M) at 60, 90, 120, 180, 240, 300, and 420 s. The plasma was separated by centrifugation and extracted with 4 volumes of ethanol. The ethanol phase was evaporated under nitrogen and redissolved in H_2O for bioassay on a guinea-pig ileum. Ventilatory pressure is given in cmH_2O and SRS-A as equivalents of 10^{-8} M leukotriene E_4, $n = 5$.

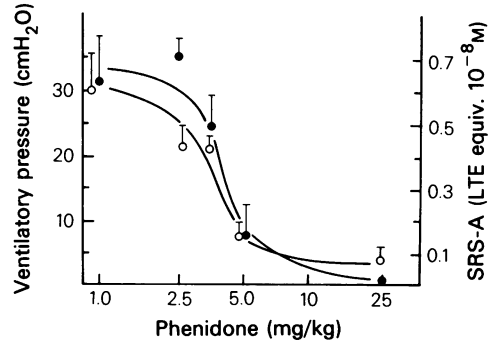

Figure 7 Effects of phenidone on antigen-induced increases in ventilatory pressure (●) and plasma SRS-A levels (○). Surgically prepared animals pretreated with indomethacin (10 mg/kg), propranolol (0.1 mg/kg), pyrilamine (2.0 mg/kg) and succinylcholine (1.2 mg/kg) were challenged with ovalbumin (1.0 mg/kg) in the presence of phenidone (1.0–25 mg/kg). Ventilatory pressure measured in cmH_2O. Blood was withdrawn 5 min following antigen challenge and extracted with ethanol. The ethanol phase was evaporated and reconstituted in H_2O for bioassay. SRS-A is presented as the mean leukotriene E_4 equivalent, $n = 4–9$; vertical line shows s.e.mean.

same animals. SRS-A, as equivalents of a standard dose of synthetic LTE_4 (10^{-8} M), was detected at 60 s, reached a maximum by 180 s and remained relatively constant through 420 s. The biologically active material extracted from the plasma and bioassayed was completely blocked by FPL 55712 (10^{-6} M) substantiating the SRS-A nature of this material.

To determine the effectiveness of the model for detecting SRS-A synthesis inhibitors, the ability of phenidone (1.0–25.0 mg/kg, i.v.) to inhibit antigen-induced increases in ventilatory pressure and increases in plasma SRS-A levels was determined. As shown in Figure 7, phenidone, in a dose-dependent manner, inhibited the antigen-induced increase in ventilatory pressure with an ED_{50} of approximately 4 mg/kg. Coincident with these effects was the dose-dependent reduction of plasma SRS-A, also with an ED_{50} of 4 mg/kg. Extracted plasma samples from animals treated with phenidone (25 mg/kg) but not challenged with antigen, showed no inhibitory activity in the bioassay towards a standard LTE_4-induced ileum contraction.

As a further control, blood was withdrawn 5 min after antigen challenge from animals treated with and without 10 mg/kg FPL 55712. This dose was previously demonstrated to inhibit completely increases in ventilatory pressure. Under these conditions, FPL 55712 had no significant ($P > 0.05$) effect on the antigen-induced plasma levels of SRS-A, as compared to animals not receiving FPL 55712.

Discussion

Since the early observations of Kellaway & Trethewie (1940) that immunological challenge of sensitized guinea-pig lung released SRS-A, this mediator has been implicated as having a major role in the pathophysiology of bronchical asthma (Orange & Austen, 1969; Austen, 1978; Sirois & Borgeat, 1980; Goetzl, 1980). Release of SRS-A from human lung tissue has also been demonstrated (Austen & Orange, 1975; Hutchcroft & Orange, 1980; Armour *et al.*, 1981), and the leukotriene components of SRS-A are potent bronchoconstrictors of human airway tissue (Collier & Sweatman, 1968; Dahlen, Hedqvist, Hammarstrom & Sammuelsson,1980; Hanna, Bach, Pare & Schellenberg, 1981). Thus, the design of pharmacological agents which can modulate the actions of the leukotrienes through synthesis inhibition, may provide additional therapeutic agents for the treatment of asthma. While many *in vitro* systems have been described for the detection of SRS-A synthesis inhibitors (Hitchcock, 1978; Orning & Hammarstrom, 1980; Patterson *et al.*, 1981; Armour *et al*, 1981) only two *in vivo* animal models have been described (Ritchie *et al.*, 1981; Blumenthal, Dervinis & Lewis, 1981). While these models are similar in nature to ours, one of these models was incapable of detecting lipoxygenase inhibitors (Blumenthal *et al.*, 1981) and none of the reported SRS-A synthesis inhibitors were tried in the other model (Ritchie *et al.*, 1981). Thus the only criterion used in these models to characterize the response obtained upon antigen challenge as being mediated by SRS-A was the ability of FPL 55712 to antagonize the response. We felt that additional criteria were necessary.

The strategy used in the design of the model described in this paper is two fold. First, the synthesis of arachidonic acid is directed into the lipoxygenase pathway by inhibition of the cyclo-oxygenase enzymatic pathway with indomethacin. Recent reports have demonstrated that guinea-pig lung tissue *in vitro* can release SRS-A (Hitchcock, 1978; Engineer, Niederhauser, Piper & Sirois, 1978; Piper & Temple, 1981) and that inhibition of cyclo-oxygenase with indomethacin can augment this release (Hitchcock, 1978; Engineer *et al.*, 1978). Secondly, the sensitivity of the airway tissue to SRS-A was increased. This involved: (1) eliminating the bronchoconstrictor effects of histamine by pretreating with the antihistamine, pyrilamine maleate and (2) enhancing the response of the guinea-pig to SRS-A with propranolol, as was previously demonstrated with exogenous LTE_4 (Welton *et al.*, 1981). Under these conditions, antigen administration resulted in a slowly developing bronchoconstriction, with a time course identical to that observed by Ritchie *et al.* (1981) in their *in vivo* model.

Previous investigators have attempted to localize the airways involved in allergic bronchoconstriction by comparing relative effects of pharmacological mediators on lung compliance and resistance (Colebatch, Olsen & Nadel, 1966; Macklem & Mead, 1967; Drazen & Austen, 1974). Theoretically, separating the probable site of an airway response is based on the premise that changes in compliance reflect involvement of small airways and changes in resistance reflect involvement of large airways. Utilizing this theory, Stein, Schiavi, Ottenberg & Hamilton (1961) and Richardson, Hogg, Bouchard & Hall (1973) demonstrated that anaphylactic bronchoconstriction in the guinea-pig is predominantly a small airway effect.

In our studies, exogenously released SRS-A produced a more drastic change in dynamic lung compliance than pulmonary resistance, indicating preferential involvement of small airways. These results are in accord with experiments reported by Drazen, Austen, Lewis, Clark, Goto, Marfat & Corey (1980) with exogenously administered leukotrienes.

Since it is well–established that FPL 55712 is an antagonist of SRS-A and leukotrienes *in vitro* (Augstein *et al.*, 1973; Drazen *et al.*, 1980; Welton *et al.*,

1981), it was considered important to determine whether FPL 55712 would block bronchoconstriction induced in our model by endogenously released leukotrienes *in vivo*. As shown by our results, FPL 55712 (10 mg/kg, i.v.) attenuated the severity of antigen-induced pulmonary anaphylaxis in sensitized guinea-pigs pretreated with indomethacin, propranolol and pyrilamine. However, when pyrilamine was omitted, FPL 55712 did not abolish antigen-induced pulmonary anaphylaxis in sensitized guinea-pigs. Thus, while an antihistamine can unmask an SRS-A component, SRS-A and histamine do not appear to be acting additively. Furthermore, histamine alone is capable of mediating a maximum increase in ventilatory pressure. These results also suggest that FPL 55712 *in vivo*, does not inhibit IgG-dependent mediator release in guinea-pigs.

FPL 55712 *in vitro* has also been shown to have effects other than SRS-A antagonism, such as inhibition of both histamine release and thromboxane synthase (Welton, Hope, Tobias & Hamilton, 1981). Our goal was to establish by additional means, that this antigen-induced response in the guinea-pig *in vivo* was mediated by SRS-A. Five reported SRS-A synthesis inhibitors were tested for the ability to inhibit bronchoconstriction. Phenidone, BW 775C and NDGA showed significant inhibition of the bronchoconstriction, while ETYA and benoxaprofen were ineffective under the conditions tested.

In dispersed pig lung cells, phenidone and ETYA were approximately equipotent in inhibiting SRS-A release with ED_{50}s of 3.5×10^{-5} M and 1.5×10^{-5} M respectively (Patterson *et al.*, 1981). ETYA was also shown to be effective in decreasing SRS-A release from chopped guinea-pig lung (Hitchcock, 1978). However, when tested in our model *in vivo*, ETYA was not effective. One explanation for this discrepancy may be related to the tight binding to albumin which occurs with fatty acids *in vivo*. BW 755C and NDGA which were equipotent in our model were also equipotent in inhibiting SRS-A release from human lung *in vitro* (Armour *et al.*, 1981). However, we must emphasize that the conditions under which these compounds were tested in our model *in vivo* were not optimized. All compounds were tested at 10 mg/kg (i.v.) with a 1 min pretreatment. Complete dose-response curves under optimal conditions would have to be established, as well as time course studies, before the relative effectiveness of a compound could be determined. Thus, by three criteria, (1) SRS-A antagonism with FPL 55712, (2) SRS-A synthesis inhibition with phenidone, BW 755C and NDGA and (3) determination of plasma SRS-A, we have demonstrated that this antigen-induced anaphylaxis following pharmacological modulation is mediated by SRS-A. In addition, the ability to detect SRS-A-like activity in arterial plasma allows for the distinction between SRS-A synthesis inhibitors and SRS-A antagonists. It is hoped that this model will be useful for the pharmacological study of modulators of the SRS-A (leukotriene) pathway.

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