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EFFECTS OF INHIBITORS OF ANAPHYLACTIC MEDIATORS IN TWO MODELS OF BRONCHIAL ANAPHYLAXIS IN ANAESTHETIZED GUINEA-PIGS

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1 The effects of pretreatment with various inhibitors of anaphylactic mediators on antigen-induced bronchoconstriction were studied in anaesthetized guinea-pigs, actively sensitized according to two different regimens (one producing IgE- and IgG-like antibodies and the other producing exclusively IgG antibodies).

2 The phospholipase A_2-inhibitors mepacrine and *p*-bromphenacylbromide caused a dose-dependent inhibition of the antigen-induced bronchoconstriction in guinea-pigs sensitized to produce both IgE and IgG antibodies. No effect was seen in those sensitized to produce only IgG antibodies.

3 In both models indomethacin pretreatment led to an increased anaphylactic bronchoreactivity, whereas mepyramine and FPL 55712 reduced it.

4 BW 755C significantly reduced antigen-induced bronchoconstriction in guinea-pigs sensitized to produce both IgE and IgG antibodies. In this model, the residual bronchoconstriction evident after combined pretreatment with indomethacin and mepyramine was prevented by additional pretreatment with mepacrine, FPL 55712 or budesonide.

5 Arachidonic acid given intravenously caused a marked bronchoconstriction that was prevented by indomethacin but not by budesonide, FPL 55712 or *p*-bromphenacylbromide.

6 Although the same pattern of anaphylactic mediators is released in the two models of anaphylactic bronchoconstriction, a different activation mechanism is indicated by the results obtained with phospholipase A_2 inhibitors.

Introduction

IgE antibody-mediated reactions may play a major role in the pathogenesis of human asthma. The reagin attaches to the surface of cells, notably mast cells and blood basophils, and interaction between it and the antigen leads to the release of mediators causing bronchial muscle contraction and inflammatory reactions (Ishizaka & Ishizaka, 1978; Johansson & Foucard, 1978; Lewis & Austen, 1981).

Andersson (1980a) showed that guinea-pigs sensitized with low doses of antigen together with $Al(OH)_3$ as an adjuvant produce both IgE- and IgG-like antibodies, whereas those sensitized with high doses of antigen without adjuvant produce only IgG-like antibodies. Furthermore, some important differences were shown between the anaphylactic reactions obtained in animals sensitized according to these two regimens. Thus higher provocation doses are needed to obtain an effect and much more histamine is released after anaphylactic challenge in guinea-pigs sensitized to produce a mixture of IgG and IgE antibodies compared with those sensitized to produce only IgG antibodies (Andersson, 1980a; Andersson & Bergstrand, 1981). Furthermore, disodium cromoglycate (SCG) and glucocorticoids do not prevent IgG-mediated bronchial anaphylaxis (Andersson, 1980b; Andersson & Bergstrand, 1981; Andersson & Brattsand, 1982).

It is therefore of interest to compare the role of different mediators in bronchial anaphylaxis mediated by IgE or IgG antibodies. Thus in the present paper the roles of histamine and the lipoxygenase and cyclo-oxygenase products of arachidonic acid metabolism are examined by the use of known antagonists in an antigen-induced bronchial anaphylaxis in guinea-pigs sensitized to produce either IgE or IgG antibodies.

Methods

Animals

Outbred guinea-pigs (Dunkin-Hartley bred by Sahlins, Malmö, Sweden) were used; their weight at sensitization was 250–300 g.

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Sensitization procedures

Two sensitization procedures (A, B) were used. (A) Production of IgE- and IgG-like antibodies: the animals were sensitized by one intraperitoneal injection of 0.5 ml 0.9% w/v NaCl solution (saline) containing 1 μg ovalbumin and 100 mg $Al(OH)_3$. The adjuvant was added to the antigen solution 1 h before injection. (B) Production of IgG-like antibodies: the animals were injected intraperitoneally with ovalbumin 5 mg on day 0 and 10 mg on day 2. The injection volume was 0.1 ml.

Details of types and titres of antibodies produced in guinea-pigs sensitized according to procedures (A) and (B) have been given before (Andersson, 1980a).

Respiratory measurements

The respiratory measurements were performed as described earlier (Andersson & Bergstrand, 1981). Guinea-pigs actively sensitized according to procedures (A) and (B) were anaesthetized with pentobarbitone (Mebumal), 30 mg/kg i.p., and challenged on day 42 with ovalbumin injected intravenously (doses indicated in Results) through the left jugular vein. When a stable response was reached, 1000 μg/kg of ovalbumin was injected to produce maximum response. Blood pressure was recorded throughout the entire procedure via a catheter inserted in the right carotid artery. The variables used to measure pulmonary mechanics and bronchoconstriction were lung resistance (R_L) and dynamic lung compliance (C_{Dyn}) as described previously. R_L and C_{Dyn} have been defined and calculated as outlined by Amdur & Mead (1958).

Drugs

Ovalbumin (grade III), indomethacin, arachidonic acid and mepacrine (Quinacrine), were from Sigma. Other drugs were mepyramine maleate, May & Baker Ltd., FPL 55712 (sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate), Fisons; *p*-bromphenacylbromide, Draco, Sweden; budesonide (16,17-(22R.S.)-propylmethylenedioxy-pregna-, 4-diene-11, 21-diol-3, 20-dione) micronized, Astra, Sweden; BW755C,

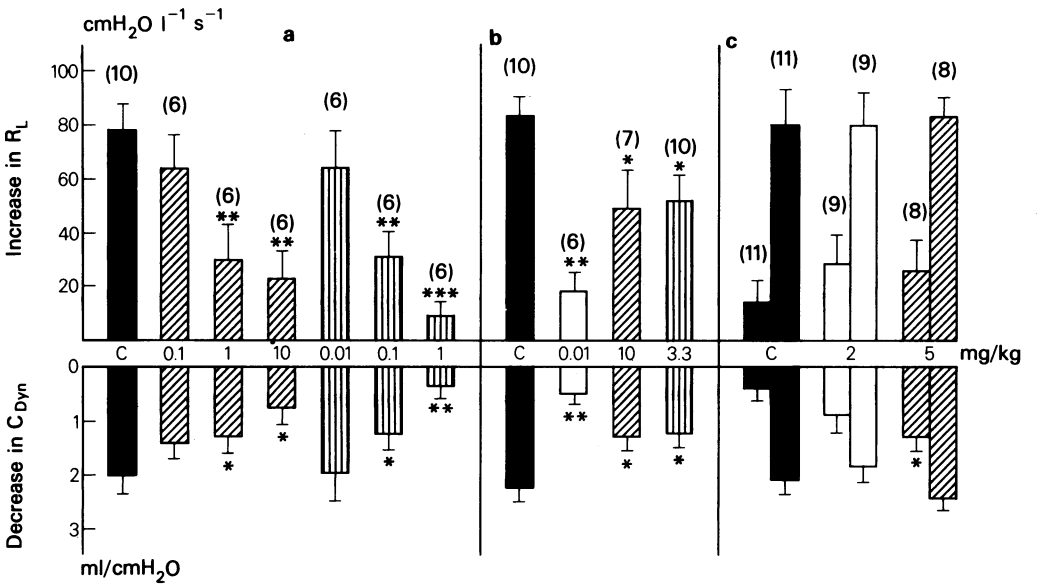

Figure 1 Effect of inhibitors of phospholipase A_2, cyclo-oxygenase, lipoxygenase, histamine and SRS-A on the antigen-induced bronchoconstriction in anaesthetized guinea-pigs sensitized to produce both IgE- and IgG-like antibodies (procedure(A)). (a) Solid columns control response to ovalbumin challenge (5 μg/kg); hatched columns dose-response for mepacrine given 5 min before challenge; striped columns dose-response for *p*-bromphenacylbromide given 5 min before challenge. (b) Solid columns control response to ovalbumin (5 μg/kg); open columns mepyramine (0.01 mg/kg) given 5 min before challenge; hatched columns FPL 55712 (10 mg/kg) given immediately before challenge; striped columns BW 755C (3.3 mg/kg) given 2 min before challenge. (c) Solid columns control responses to ovalbumin (2 and 5 μg/kg); indomethacin, 2 mg/kg (open columns) and 5 mg/kg (hatched columns) given 5 min before challenge. Columns represent mean results, bars show s.e.mean: figures in parentheses indicate the number of experiments. Statistical significance of differences is shown with respect to control anaphylaxis: $*0.05 > P > 0.01$ $**0.01 > P > 0.001$ $***P < 0.001$.

Wellcome Research Laboratories. Budesonide was suspended in a vehicle of carboxymethyl cellulose-sodium 0.75%, Tween 80 0.4% and NaCl 0.7%. Indomethacin and *p*-bromphenacylbromide were dissolved in propylene glycol. The other compounds were dissolved and diluted in saline.

Potential inhibitors of anaphylactic bronchoconstriction were administered at various times: FPL 55712 was given immediately before challenge; BW 755C was given intravenously 2 min before challenge; mepacrine, *p*-bromphenacylbromide, indomethacin and mepyramine were all given intravenously 5 min before challenge and budesonide was given intraperitoneally 15–20 h before challenge.

Statistics

The statistical significance of differences was evaluated by Student's unpaired *t* test.

Results

Effects of inhibitors on IgE/IgG-mediated anaphylactic bronchoconstriction

Guinea-pigs were sensitized by procedure (A) which results in the production of both IgE- and IgG-like antibodies. Anaphylactic challenge on day 42 resulted in bronchoconstriction in the anaesthetized animals, and this could be partially prevented by pretreatment with various inhibitors (Figure 1). Mepacrine (0.1–10 mg/kg) and *p*-bromphenacylbromide (0.01–1 mg/kg) both caused dose-related inhibition of the antigen-induced bronchoconstriction as measured in terms of lung resistance or lung compliance (Figure 1a). Mepyramine (0.01 mg/kg) caused a marked inhibition of the antigen-induced bronchoconstriction (Figure 1b). Indomethacin (2–5 mg/kg) caused a small dose-related increase in bronchoconstriction when low but not when large provocation doses were used (Figure 1c). FPL55712 (10 mg/kg) and BW755C (3.3 mg/kg) both caused partial inhibition of the antigen-induced bronchoconstriction (Figure 1b). However, BW755C at 10 mg/kg caused pronounced bronchospasm (results not shown).

Effects of inhibitors on anaphylactic bronchoconstriction mediated solely by IgG-like antibodies

Animals were sensitized according to procedure (B) and results are shown in Figure 2. *p*-Bromphenacylbromide (1 mg/kg) had no effect on

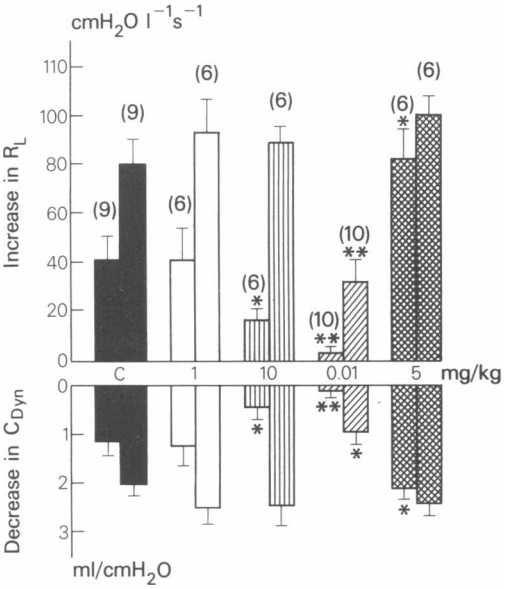

Figure 2 Effect of inhibitors of phospholipase A_2, SRS-A, histamine and cyclo-oxygenase on the antigen-induced bronchoconstriction in anaesthetized guinea-pigs sensitized to produce IgG antibodies (procedure (B)). Solid columns control responses to ovalbumin challenge (40 and 120 μg/kg); open columns *p*-bromphenacylbromide (1 mg/kg) given 5 min before challenge; striped column FPL 55712 (10 mg/kg) given immediately before challenge; hatched columns mepyramine (0.01 mg/kg) given 5 min before challenge; cross-hatched columns indomethacin (5 mg/kg) given 5 min before challenge.
Columns represent mean results, bars show s.e.mean: figures in parentheses indicate the number of experiments. Statistical significance of differences is shown with respect to control anaphylaxis: $*0.05 > P > 0.01$; $**0.01 > P > 0.001$.

this type of antigen-induced bronchoconstriction. FPL 55712 (10 mg/kg) caused a marked reduction of the antigen-induced bronchospasm when small but not when large provocation doses were used. Indomethacin (5 mg/kg) caused a weak enhancement in anaphylactic reactivity. Mepyramine 0.01 mg/kg markedly decreased the antigen-induced bronchospasm.

Effects of inhibitors on IgE/IgG-mediated anaphylactic bronchoconstriction after indomethacin and mepyramine pretreatment

Whereas mepyramine alone caused marked inhibition of this type of antigen-induced bronchoconstriction (Figure 1b), combined pretreatment with mepyramine and indomethacin resulted in bronchoconstriction of greater intensity, although less

than in untreated animals (R_L of 54.9±6.2 versus 79.7±8.9, $P<0.01$; compare Figure 3 with Figure 1a). In untreated guinea-pigs the bronchospasm began with 20–30 s and was maximal 2–3 min after antigen administration. With mepyramine-indomethacin pretreatment, the contraction began to develop 2–3 min after antigen injection and reached maximum 6–10 min later. This bronchospasm of longer latency was dose-dependently inhibited by FPL 55712 and mepacrine (Figure 3). Budesonide pretreatment (50 mg/kg) also caused partial inhibition of the bronchoconstriction.

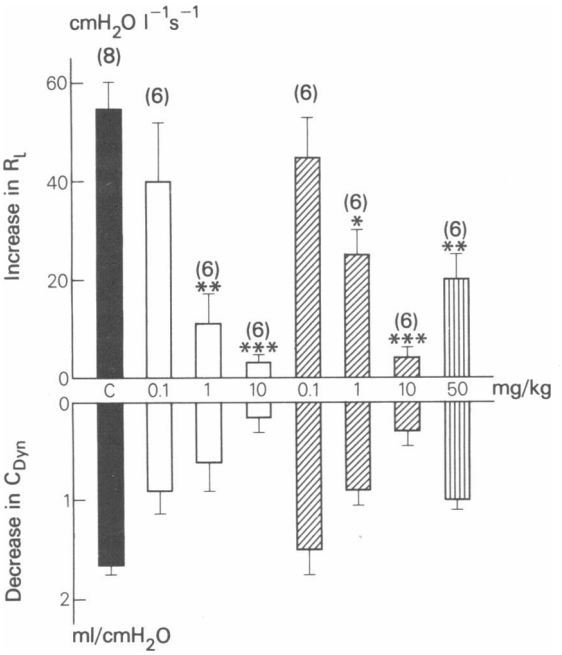

Figure 3 Effects of inhibitors of phospholipase A_2 and SRS-A and of budesonide on the antigen-induced bronchoconstriction in anaesthetized guinea-pigs sensitized to produce both IgE- and IgG-like antibodies and pretreated with indomethacin and mepyramine. Solid columns, control response to ovalbumin (5 μg/kg), mepyramine (0.01 mg/kg) and indomethacin (5 mg/kg) given 5 min before challenge; open columns, dose-response for mepacrine given 5 min before challenge; hatched columns, dose-response for FPL 55712 given immediately before challenge; striped columns, budesonide (50 mg/kg) given 15–20 h before challenge. Columns represent mean results, bars show s.e.mean: figures in parentheses indicate the number of experiments. Statistical significance of differences is shown with respect to control anaphylaxis: $*0.05>P>0.01$; $**0.01>P>0.01$; $***P<0.001$.

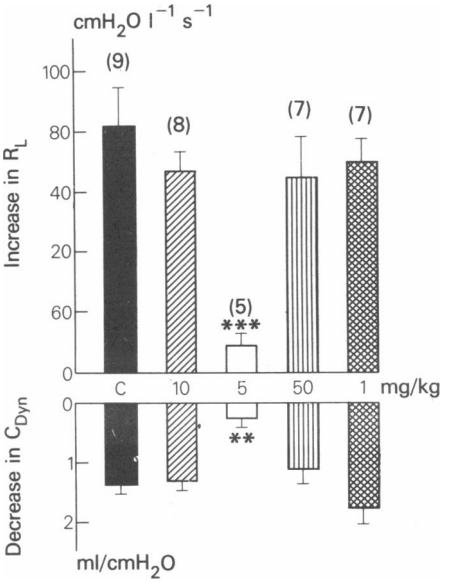

Figure 4 Effect of inhibitors of phospholipase A_2, cyclo-oxygenase and SRS-A and of budesonide on bronchoconstriction induced by arachidonic acid in anaesthetized guinea-pigs. Solid columns, control response to arachidonic acid (10 mg/kg); hatched columns, FPL 55712 (10 mg/kg) given immediately before challenge; open columns, indomethacin (5 mg/kg) given 5 min before challenge; striped columns, budesonide (50 mg/kg) given 15–20 h before challenge; cross-hatched columns, *p*-bromphenacylbromide (1 mg/kg) given 5 min before challenge. Columns represent mean results, bars show s.e.mean. Figures in parentheses indicate the number of experiments. Statistical significance of differences is shown with respect to control anaphylaxis: $**0.01>P>0.001$; $***P<0.001$.

Effects of inhibitors on arachidonic acid-induced bronchospasm in unsensitized guinea-pigs

Arachidonic acid (10 mg/kg) given intravenously caused a rapidly developing bronchospasm which was unaffected by pretreatment with budesonide (50 mg/kg), FPL 55712 (10 mg/kg) or *p*-bromphenacylbromide (1 mg/kg), but almost completely inhibited by indomethacin (5 mg/kg) (Figure 4).

Discussion

IgE antibodies attach themselves to the surface of mast cells and blood basophil leukocytes, and the subsequent interaction between antigen and antibody leads to the release of mediators participating in the anaphylactic reaction. During the last few years, understanding of the mechanisms behind the release

of anaphylactic mediators triggered by IgE antibodies has increased. With rat mast cells or human basophils it has been shown that bridging of IgE receptors induces activation of methyltransferases which cause a series of phospholipid methylations. Production of cyclic AMP occurs simultaneously (Ishizaka, 1981). The stimulation of phospholipid methylation at the plasma membrane results in an increase in Ca^{2+} influx concomitant with histamine release. In this process the Ca^{2+}-dependent phospholipase A_2 seems to play an important role. Treatment of rat basophilic leukaemia cells or human basophils with mepacrine or *p*-bromphenacylbromide, compounds which block phospholipase A_2 in various systems (Volwerk, Pieterson & De Haas, 1974; Vargaftig, 1977; Blackwell, Flower, Nijkamp & Vane, 1978), results in a decreased antigenor anti-IgE-induced histamine release (McGiveny, Morita, Crews, Hirata, Axelrod & Siraganian, 1980; Marone, Kagey-Sobotka & Lichtenstein, 1981).

Activation of purified rat mast cells and rat basophil leukaemia cells by antigen or anti-IgE also leads to the release of arachidonic acid from membrane phospholipids, by action of phospholipase A_2 (Hirata & Axelrod, 1980). Arachidonic acid is metabolized through the cyclo-oxygenase pathway into prostacyclins, prostaglandins and thromboxanes or through the lipoxygenase pathway leading to production of SRS-A (Lewis & Austen, 1981). Recent research has led to the identification of the principal active constituents of SRS-A, leukotrienes C_4 and D_4 (Bach, Brashler, Hammarström & Samuelsson, 1980; Borgeat, 1981).

SRS-A has long been known to take part in type I allergic reactions (Piper, 1977; Borgeat, 1981; Lewis & Austen, 1981). Early observations indicated effects on airway smooth muscle contractility both *in vivo* and *in vitro*. During the last few years, the compound FPL 55712, an antagonist of SRS-A, has been used in several immunopharmacological studies to define the role of SRS-A in anaphylactic reactions (Chand, 1979). The fact that FPL 55712 has a very short biological half-life and has to be given almost immediately before challenge poses great difficulties in its use in studies *in vivo*. However, high doses of FPL 55712 given immediately before antigen challenge have been shown to block SRS-A-induced bronchospasm in guinea-pigs (Sheard, Lee & Tattersall, 1977). Furthermore, FPL 55712 given as an aerosol inhibits bronchoconstriction induced by nebulized LTC_4 and LTD_4 in man (Holroyde, Altounyan, Cole, Dizon & Elliot, 1981).

The results obtained in the present investigation show that the phospholipase A_2 inhibitors mepacrine or *p*-bromphenacylbromide inhibit the IgE- but not the IgG-mediated bronchoconstriction. The role of histamine in both the IgE- and IgG-mediated anaphylaxis is indicated by the blockade with the H_1-antagonist, mepyramine.

The present investigation also shows that FPL 55712 to some extent inhibited the antigen-induced bronchospasm in guinea-pigs sensitized to produce both IgE and IgG antibodies. Furthermore, the participation of lipoxygenase-derived products in the IgE-mediated bronchospasm was strengthened by the use of BW 755C (3.3 mg/kg), a compound mainly acting as a lipoxygenase inhibitor (Higgs, Flower & Vane, 1979). A higher dose (10 mg/kg) of BW 755C caused contraction. This effect might depend on the ability of the compound to induce histamine release (Patterson, Pruzansky & Harris, 1981). These results indicate that both SRS-A and histamine contribute to the IgE- and IgG-mediated bronchoconstriction in anaesthetized guinea-pigs.

The role of the products derived by the cyclo-oxygenase pathway in the anaphylactic lung reaction is controversial. There are reports of antigen-induced production of prostaglandins and thromboxanes in various species including man and guinea-pig (Piper, 1977; Al Ubaidi & Bakhle, 1980; Spannhake, Hyman & Kadowitz, 1981). However, it has been observed that the cyclo-oxygenase inhibitor indomethacin has no effect on the antigen-induced bronchoconstriction in the guinea-pig (Michoud, Frazer, Pare & Hogg, 1976). Furthermore, asthmatic patients show no reduction of asthmatic symptoms after indomethacin pretreatment (Smith, 1975).

In the present investigation, indomethacin has a strong blocking action on the bronchospasm induced by arachidonic acid infusion, suggesting that such a bronchospasm is induced by thromboxane A_2 or contracting prostaglandins. However, indomethacin causes a potentiation of the antigen-induced IgE- and IgG-mediated bronchoconstriction. These results indicate that the antigen-induced bronchospasm in the guinea-pig is not due to cyclo-oxygenase-derived products. Although it was originally suggested that the potentiating effect of indomethacin was due to inhibition of formation of cyclo-oxygenase products like prostacyclin or prostaglandin E_2 which have been shown to inhibit mediator release or to relax tracheal smooth muscle contracted by histamine (Lichtenstein & Henney, 1972; Tauber, Kaliner, Stechschulte & Austen, 1973; Orehek, Douglas, Lewis & Bouhuys, 1973; Engineer, Jose, Piper & Tippins, 1978), more recent evidence indicates that the potentiating effect exerted by indomethacin is related to a redirection of arachidonic acid metabolism through the lipoxygenase pathway (Hamberg, 1976; Morris, Piper, Taylor & Tippins, 1980). This may result in increased production of 5-HPETE which has been shown to enhance histamine release from human basophils (Peters, Siegel, Kagey-Sobotka & Lichtenstein, 1981) and/or to in-

creased production of SRS-A (Piper, Tippins, Morris & Taylor, 1979). The results obtained in the present investigation show that antigen administration to sensitized guinea-pigs pretreated with mepyramine and indomethacin results in a marked bronchospasm which is inhibited by FPL 55712, the glucocorticoid budesonide and mepacrine, at least indicating production of SRS-A.

The results obtained in the model for IgE-mediated bronchoconstriction seem to fit into the theories reviewed by Hirata & Axelrod (1980) and Ishizaka (1981). The anaphylactic response mediated by antibodies of the IgG-type has been far less investigated. There are reports of anaphylactic antibodies of the IgG-type in asthmatics (Bryant, Burns & Lazarus, 1973; 1975). However, the pathophysiological importance of the IgG antibody-mediated reactions for human asthma is not clear (Ishizaka & Ishizaka, 1973).

As there are reports of IgG-mediated release of anaphylactic mediators like histamine and SRS-A from rat peritoneal mast cells (Moodley & Mongar, 1981) and guinea-pig lung *in vitro* (Andersson & Bergstrand, 1981; Forsberg & Sörenby, 1981), it seems reasonable to assume the release of histamine and SRS-A induced by IgG antibodies in the present investigation to be derived from mast cells. There are indications of separate receptors for IgG and IgE antibodies on mice mast cells (Daëron, Prouvost-Danon & Voisin, 1980). This observation together with the findings of different effects with proposed phospholipase A_2 inhibitors in the present investigation, indicate involvement of different activation mechanisms in the IgE- and IgG-mediated anaphylactic reaction.

In conclusion, the present investigation shows that histamine and SRS-A are the main mediators of antigen-induced bronchospasm in guinea-pigs sensitized to produce IgE and/or IgG antibodies, whereas products derived via the cyclo-oxygenase pathways of the arachidonic acid metabolism contribute to a lesser extent to the bronchospasm. An antigen-induced, SRS-A-mediated bronchospasm can be obtained in mepyramine-indomethacin-treated guinea-pigs sensitized to produce both IgE and IgG antibodies. This bronchospasm is inhibited by the phospholipase A_2 inhibitors, mepacrine and budesonide. The findings that pretreatment with phospholipase A_2 inhibitors decreased the IgE- but not the IgC-mediated bronchoconstriction indicate different activation mechanisms for the release process induced by IgE and IgG antibodies.

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References

AL-UBAIDI, F. & BAKHLE, Y.S. (1980). Differences in biological activation of arachidonic acid in perfused lungs from guinea-pig, rat and man. *Eur. J. Pharmac.*, **62**, 89–96.

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

ANDERSSON, P. (1980a). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Pattern of response in relation to immunization regimen. *Allergy*, **35**, 65–71.

ANDERSSON, P. (1980b). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Anti-anaphylactic effects of sodium cromoglycate and aminophylline. *Br. J. Pharmac.*, **69**, 467–472.

ANDERSSON, P. & BERGSTRAND, H. (1981). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: effect of long-term treatment with sodium cromoglycate and aminophylline. *Br. J. Pharmac.*, **74**, 601–609.

ANDERSSON, P. & BRATTSAND, R. (1982). Protective effects of the glucocorticoid budesonide on lung anaphylaxis in actively sensitized guinea-pigs: Inhibition of IgE- but not of IgG- mediated anaphylaxis. *Br. J. Pharmac.*, **76**, 139–147.

BACH, M.K., BRASHLER, J.R., HAMMARSTRÖM, S. & SAMUELSSON, B. (1980). Identification of a component of rat mononuclear cell SRS as leukotriene D. *Biochem. biophys. Res. Comm.*, **93**, 1121–1126.

BLACKWELL, G.J., FLOWER, R.J., NIJKAMP, F.P. & VANE, J.R. (1978). Phospholipase A_2 activity of guinea-pig isolated perfused lungs: stimulation, and inhibition by anti-inflammatory steroids. *Br. J. Pharmac.*, **62**, 79–89.

BORGEAT, P. (1981). Leukotrienes: a major step in the understanding of immediate hypersensitivity reactions. *J. med. Chem.*, **24**, 121–126.

BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1973). New type of allergic asthma due to IgG "reaginic" antibody. *Br. med. J.*, **4**, 589–592.

BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1975). Identification of IgG antibody as a carrier of reaginic activity in asthmatic patients. *J. Allergy Clin. Immunol.*, **56**, 417–428.

CHAND, N. (1979). FPL 55712 – an antagonist of slow reacting substance of anaphylaxis (SRS-A): a review. *Agents & Actions*, **9**, 133–140.

DAËRON, M., PROUVOST-DANON, A. & VOISIN, G.A. (1980). Mast cell membrane antigens and Fc receptors in anaphylaxis II. Functionally distinct receptors for IgG and for IgE on mouse mast cells. *Cell Immunol.*, **49**, 178–189.

ENGINEER, D.M., JOSE, P.J., PIPER, P.J. & TIPPINS, J.R. (1978). Modulation of slow-reacting substance of anaphylaxis and histamine release by prostacyclin and thromboxanes. *J. Physiol.*, **281**, 42P.

FORSBERG, K. & SÖRENBY, L. (1981). The influence of a new coricosteroid, budesonide, on anaphylactic bronchoconstriction and SRS-A release in the guinea-pig. *Agents & Actions*, **11**, 391–395.

HAMBERG, M. (1976). On the formation of thromboxane B_2 and 12L-hydroxy 5,8,10,14-eicosatetraenoic acid (12 ho-20:4) in tissues from the guinea-pig. *Biochem. biophys. Acta.*, **431**, 651–654.

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

HIRATA, F. & AXELROD, J. (1980). Phospholipid methylation and biological signal transmission. *Science*, **209**, 1082–1090.

HOLROYDE, M.C., ALTOUNYAN, R.E.C., COLE, M., DIXON, M. & ELLIOTT, E.V. (1981). Bronchoconstriction produced in man by leukotrienes C and D. *Lancet*, **ii**, 17–18.

ISHIZAKA, T. (1981). Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. *J. Allergy Clin. Immunol.*, **67**, 90–96.

ISHIZAKA, K. & ISHIZAKA, T. (1973). Role of IgE and IgG antibodies in reaginic hypersensitivity in the respiratory tract. In *Asthma: Physiology, Immunopharmacology and Treatment.* ed. Austen, K.F., Lichtenstein, L.M. pp. 55–70. New York & London: Academic Press.

ISHIZAKA, K. & ISHIZAKA, T. (1978). Immunology of IgE-mediated hypersensitivity. In *Allergy: Principles and Practice.* ed. Middleton Jr., E., Read, C.E. & Ellis, E.F. pp. 52–78. Saint Louis, U.S.A.: C.V. Mosby CO.

JOHANSSON, S.G.O. & FOUCARD, T. (1978). IgE in immunity and disease. In *Allergy: Principles and Practice.* ed. Middleton Jr., E., Read, C.E. & Ellis, E.F. pp. 551–562. Saint Louis, U.S.A.: C.V. Mosby & Co.

LEWIS, R.A. & AUSTEN, K.F. (1981). Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. *Nature*, **293**, 103–108.

LICHTENSTEIN, L.M. & HENNEY, C.S. (1972). Prostaglandin inhibition of immediate and delayed hypersensitivity in vitro. In *Prostaglandins in Cellular Biology.* ed. Ramwell, P.W. pp. 293–308. New York: Plenum Press.

MARONE, G., KAGEY-SOBOTKA, A. & LICHTENSTEIN, L.M. (1981). Possible role of phospholipase A_2 in triggering histamine secretion from human basophils in vitro. *Clin. Immunol. Immunopath.*, **20**, 213–239.

MCGIVENY, A., MORITA, Y., CREWS, F.T., HIRATA, F., AXELROD, J. & SIRAGANIAN, R.P. (1981). Phospholipase activation in the IgE-mediated and Ca^{2+} ionophore A 23187-induced release of histamine from rat basophilic leukemia cells. *Archs biochem. Biophys.*, **212**, 572–580.

MICHOUD, M.-C., FRASER, R.S., PARE, P.D. & HOGG, J.C. (1976). Effect of indomethacin and atropine in experimental asthma in conscious guinea-pigs. *J. appl. Physiol.*, **40**, 889–894.

MOODLEY, I. & MONGAR, J.L. (1981). IgG receptors on the mast cell. *Agents & Actions*, **11**, 77–83.

MORRIS, H.R., PIPER, P.J., TAYLOR, G.W. & TIPPINS, J.R. (1980). The role of arachidonate lipoxygenase in the release of SRS-A from guinea-pig chopped lung. *Prostaglandins*, **19**, 371–383.

OREHEK, J., DOUGLAS, J.S., LEWIS, A.J. & BOUHUYS, A. (1973). Prostaglandin regulation of airway smooth muscle tone. *Nature, New Biol.*, **245**, 84–85.

PATTERSON, R., PRUZANSKY, J.J. & HARRIS, K.E. (1981). An agent that releases basophil and mast cell histamine but blocks cyclo-oxygenase and lipoxygenase metabolism of arachidonic acid inhibits immunoglobulin E-mediated asthma in rhesus monkeys. *J. Allergy Clin. Immunol.*, **67**, 444–449.

PETERS, S.P., SIEGEL, M.I., KAGEY-SOBOTKA, A. & LICHTENSTEIN, L.M. (1981). Lipoxygenase products modulate histamine release in human basophils. *Nature*, **292**, 455–457.

PIPER, P.J. (1977). Anaphylaxis and the release of active substances in the lung. *Pharmac. Ther.*, **3**, 75–98.

PIPER, P.J., TIPPINS, J.R., MORRIS, H.R. & TAYLOR, G.W. (1979). Arachidonic acid metabolism and SRS-A. *Agents & Actions*, Suppl. **4**, 37–48.

SHEARD, P., LEE, T.B. & TATTERSALL, M.L. (1977). Further studies on the SRS-A antagonist FPL 55712. *Monogr. Allergy*, **12**, 245–249.

SMITH, A.P. (1975). Effect of indomethacin in asthma: evidence against a role for prostaglandins in its pathogenesis. *Br. J. clin. Pharmac.*, **2**, 307–309.

SPANNHAKE, E.W., HYMAN, A.L. & KADOWITZ, P.J. (1981). Bronchoactive metabolites of arachidonic acid and their role in airway function. *Prostaglandins*, **22**, 1013–1026.

TAUBER, A.I., KALINER, M., STECHSCHULTE, D.J. & AUSTEN, K.F. (1973). Immunologic release of histamine and slow reacting substance of anaphylaxis from human lung. V. Effects of prostaglandins on release of histamine. *J. Immunol.*, **111**, 27–32.

VARGAFTIG, B.B. (1977). Carrageenan and thrombin trigger prostaglandin synthetase-independent aggregation of rabbit platelets: inhibition by phospholipase A_2 inhibitors. *J. Pharm. Pharmac.*, **29**, 222–228.

VOLWERK, J.J., PIETERSON, W.A. & DE HAAS, G.H. (1974). Histidine at the active site of phospholipase A_2. *Biochemistry*, **13**, 1446–1454.

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