Br. J. Pharmac. (1981), **74**, 601–609

ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN ACTIVELY SENSITIZED GUINEA-PIGS: EFFECT OF LONG-TERM TREATMENT WITH SODIUM CROMOGLYCATE AND AMINOPHYLLINE

PER ANDERSSON & HÅKAN BERGSTRAND

Research and Development Laboratories, AB Draco, Box 1707, S-221 01 Lund, Sweden

1 The effects of long-term treatment with sodium cromoglycate (SCG) and aminophylline on antigen-induced bronchoconstriction have been studied in guinea-pigs actively sensitized according to two different regimens (one producing IgE- and IgG-like antibodies and the other producing exclusively IgG-like antibodies).

2 Treatment for three weeks with SCG (10 mg/kg) and aminophylline (10, 30 or 60 mg/kg) led to a decreased bronchial response capacity which persisted even three days after treatment ceased. In this respect SCG was effective only in guinea-pigs sensitized to produce at least partly IgE-like antibodies; aminophylline was effective in guinea-pigs sensitized to produce both IgE and/or IgG antibodies.

3 The results *in vivo* with SCG were reflected *in vitro* by a reduced capacity of chopped lung tissue to release histamine at antigen challenge; lungs from animals treated with aminophylline did not show reduced histamine releasing capacity.

4 Acute treatment with atropine was shown to reduce significantly the antigen-induced bronchial contraction in guinea-pigs sensitized to produce both IgE- and IgG-antibodies. No effect of atropine was seen on an IgG-mediated anaphylaxis.

5 Increased reactivity to methacholine but not to histamine was seen in guinea-pigs sensitized to produce both IgG- and IgE-antibodies. Long-term treatment with SCG did not affect this hyper-reactivity to methacholine.

6 Decreased reactivity to isoprenaline was found in isolated tracheae taken from guinea-pigs sensitized to produce both IgE- and IgG-like antibodies compared to unsensitized guinea-pigs. Long-term treatment with SCG, but not with aminophylline, reversed this decreased reactivity.

Introduction

Bryant, Burns & Lazarus (1973) reported that sodium cromoglycate (SCG) is effective in most asthmatic patients with IgE-mediated bronchospasm, whereas it is ineffective in those with IgG-mediated bronchospasm. Inhibition of mast cell mediator release was thought to be the drug's mechanism of action. Recently, other mechanisms of action have been proposed by Harries, Parkes, Lessof & Orr (1981). These authors suggested that SCG may act on bronchial irritant receptors or directly on smooth muscle in asthmatic patients. It has been proposed that the benefit of theophylline therapy is due to its bronchodilator action. However, another action of theophylline that might be of great value in the treatment of bronchial asthma is the inhibition of mediator release (Lichtenstein & Margolis, 1968; Orange, Austen & Austen, 1971). We have previously described the characteristics of bronchial anaphylactic reactions in guinea-pigs actively sensitized to graded doses of ovalbumin (Andersson, 1980a). In an earlier investigation it was observed that a bronchial anaphylactic reaction which, at least partly, is mediated by IgE-like antibodies, is greatly inhibited by acute treatment with SCG, whereas an IgG-mediated reaction is not. Theophylline inhibits both IgG- and IgE-mediated reactions (Andersson, 1980b). However, long-term treatment with the investigated compounds would possibly be more relevant to the clinical situation. The aim of the present study was to investigate the effect of long-term treatment with SCG and theophylline in guinea-pigs sensitized to produce preferentially IgE-like or IgG-like homocytotropic antibodies.

Methods

Outbred guinea-pigs (Dunkin-Hartley) bred by

0007-1188/81/110601-09 $01.00

Sahlins, Malmö, Sweden, were used. Their weight at the time of sensitization was 250–300 g.

Sensitization procedures

Two major 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. The animals were challenged on day 63 with ovalbumin 5 μg/kg.

(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. The animals were challenged on day 63 with ovalbumin 120 μg/kg.

Respiratory measurements

The animals were anaesthetized with pentobarbitone (Mebumal, ACO, Sweden) 30 mg/kg intraperitoneally, tracheotomized and ventilated by a Braun constant volume respirator (frequency 70/min, volume 5 ml/kg). Pulmonary mechanics, i.e. lung resistance (R_L) and dynamic lung compliance (C_{Dyn}), were estimated by the Amdur & Mead (1958) method modified for anaesthetized guinea-pigs, as given below. Airflow (V) was measured by a mesh screen pneumotachograph (Fleisch no. 000) connected to a Statham differential pressure transducer (PM 15). Tidal volume (V_T) was determined as the electrical integral of airflow. The transpulmonary pressure (P_{TP}) was determined by connecting one inlet of a differential pressure transducer (Statham PM 5) to a needle inserted through the 5th or 6th intercostal space to measure interpleural pressure, and the other inlet to a small piece of rubber tubing placed between the endotracheal tube and the pneumotachograph to measure the intratracheal pressure. Output signals representing P_{TP}, V, and V_T were registered simultaneously on a Grass polygraph model 7. R_L and C_{Dyn} were determined by manual calculations according to the Amdur & Mead (1958) method. Throughout the entire experiment, blood pressure was registered by a catheter inserted in the right carotid artery.

Drug administration

SCG (10 mg/kg procedures a and b) was given as one intraperitoneal injection daily , 5 days a week for 3 weeks. Aminophylline (10, 30, and 60 mg/kg procedure (a), 30 mg/kg procedure (b)) was given orally twice a day according to the same regimen. The treatment was stopped 24 h, 3 days, or 7 days before test. Other compounds used were administered as indicated in the result section. The compounds were dissolved and diluted in saline.

Histamine release from chopped guinea-pig lung

Guinea-pigs were sensitized according to procedure (a) or (b) and underwent long-term treatment with SCG or aminophylline. The animals were stunned. The lungs were perfused through the pulmonary artery with chilled Krebs solution, removed, and immediately placed in chilled Krebs solution. They were dissected free from major airways and blood vessels, and chopped with scissors. The fragments were washed in additional Krebs solution and weighed into 100 mg portions. Each histamine release assay was performed in a total volume of 1 ml Krebs solution. To induce release, ovalbumin (final concentration 0.01, 1.0, 10.0, or 100.0 μg/ml) was added and the incubation lasted for 30 min at 37°C. The reaction was terminated by placing the tubes on ice. In all experiments, the total histamine content of the lung was extracted by boiling the fragments for 8 min after the release process. The tissue was removed from the samples and to the supernatants was added one tenth their volume of 70% PCA and precipitation of protein was carried out for 15 min. The samples were centrifuged at 800 *g* for 10 min and the histamine content of the supernatant fraction was quantified by the spectrophotofluorometric method described by May, Lyman, Alberto & Cheng (1970). Separate experiments indicated that extraction of histamine from the supernatants did not significantly influence the results. This step was therefore omitted in the present investigation. Spontaneous release from the tissue was evaluated by carrying 100 mg samples of lung tissue, in the absence of antigen, through the complete assay procedure. The percentage spontaneous histamine release was $9.8 \pm 0.7\%$ (mean ± s.e.mean, $n = 38$). The percentage histamine release was calculated according to the following formula:

$$\frac{\text{antigen-induced release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}}$$

Guinea-pig isolated trachea

The sensitivity to isoprenaline was examined in tracheae taken from unsensitized guinea-pigs or dissected out before antigen challenge from sensitized guinea-pigs undergoing respiratory measurements or chopped lung experiments. The trachea was spirally cut and placed in an organ bath filled with Krebs solution (37°C), aerated with 95% O_2 and 5% CO_2. The trachea was connected to a force displacement

transducer (FT03) and recordings were made on a Grass polygraph model 7. The initial tension was 1.5 g. The preparations were contracted with 0.3 μg/ml carbachol, and cumulative dose-response curves were obtained with isoprenaline. The effect of each increase in the dose of isoprenaline was calculated as a percentage of the maximum relaxation obtained with isoprenaline.

Drugs

The following drugs were used: ovalbumin (Sigma grade III); aminophylline (ACO, Sweden); sodium-cromoglycate (SCG, Fisons); isoprenaline (Sigma); methacholine chloride (Sigma); *o*-phthalaldehyde (BDH Chemicals Ltd); atropine sulphate (Apoteksbolaget, Sweden); carbacholine chloride (Apoteksbolaget, Sweden); histamine chloride (Apoteksbolaget, Sweden). In some experiments, $Al(OH)_3$ (F2200 Reheis Chemical Company obtained through AB Astra, Södertälje) was used as an adjuvant. The compounds used for determination of histamine and for preparation of Krebs solution were analytically pure standard chemicals. The Krebs solution contained (mM): NaCl 118, KCl 4.7, CaCl 2.5, $MgSO_4$ 1.16, $NaHCO_3$ 25, KH_2PO_4 1.18 and D-glucose 11.

Statistics

Statistical evaluation was by Student's *t* test. The dose-response curves were compared by parallel line assay (Finney, 1952).

Results

Effect of long-term treatment with sodium cromoglycate and aminophylline on antigen-induced bronchial anaphylaxis in guinea-pigs sensitized to produce both IgE- and IgG-like antibodies (Procedure a)

In guinea-pigs sensitized according to procedure (a), the pre-challenge level of R_L and C_{Dyn} was 20.4 ± 2.7 cm H_2O l^{-1} s^{-1} and 2.42 ± 0.31 ml/cmH_2O (mean ± s.e.mean, $n = 196$). The effects of treating guinea-pigs sensitized according to procedure (a) with SCG once a day or aminophylline twice a day for three weeks are summarized in Figure 1. No effect could be seen with SCG (10 mg/kg) given as a single dose when tests were performed 1, 3 or 7 days after drug administration. However, long-term treatment with SCG (10 mg/kg daily) resulted in depression of the antigen-induced bronchial anaphylaxis 1, 3 and 7

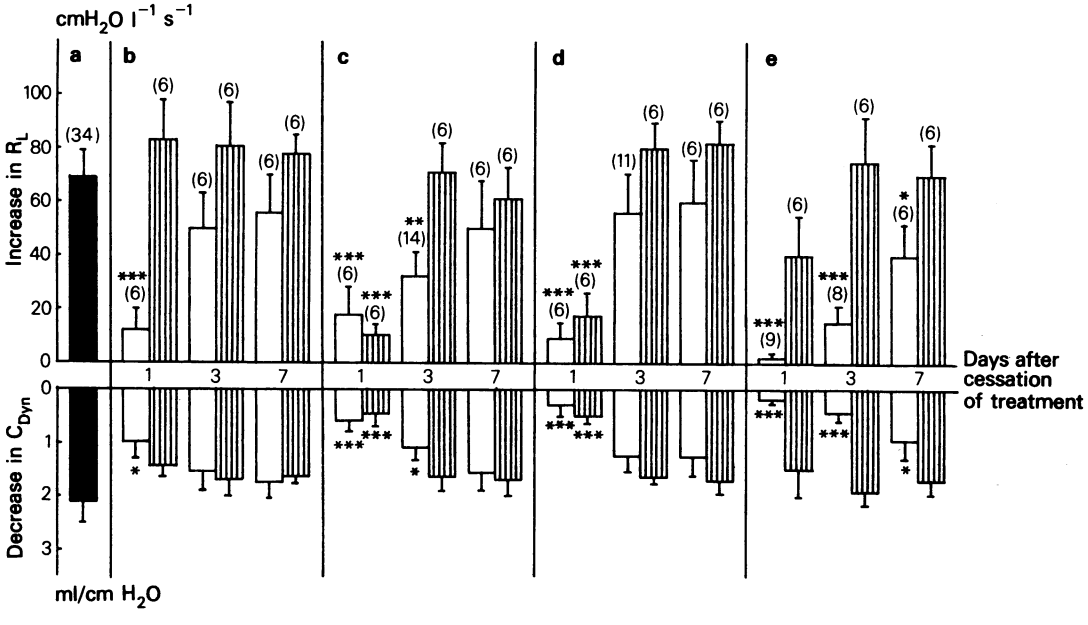

Figure 1 Increase in lung resistance (R_L) and decrease in compliance (C_{Dyn}) (mean values, vertical lines show s.e.mean) after long-term or acute treatment with sodium cromoglycate (SCG) and aminophylline in guinea-pigs actively sensitized by procedure (a): solid column control response; open column long-term treatment; striped column acute treatment. (a) Control response; (b) aminophylline 10 mg/kg; (c) aminophylline 30 mg/kg; (d) aminophylline 60 mg/kg; (e) SCG 10 mg/kg. Numbers in parentheses indicate the number of experiments. ***$P < 0.001$; *$0.05 > P > 0.01$.

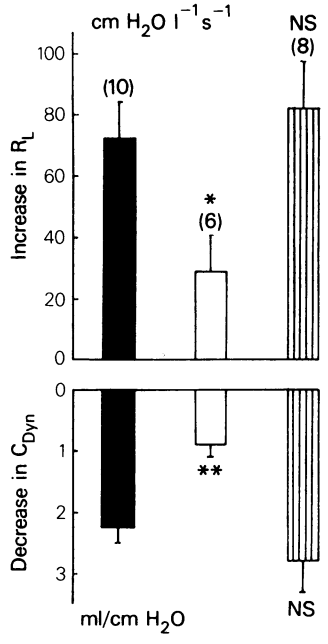

Figure 2 Increase in lung resistance (R_L) and decrease in compliance (C_{Dyn}) (mean values, vertical lines show s.e.mean) after long-term treatment with sodium cromoglycate (SCG) and aminophylline in guinea-pigs actively sensitized by procedure (b). Solid columns control response; open columns aminophylline 30 mg/kg; striped columns SCG 10 mg/kg. Number in parentheses indicate the number of experiments. NS = Not significant. *$0.05 > P > 0.01$; **$0.01 > P > 0.001$.

days after treatment ceased. As with SCG, acute treatment with aminophylline (10 mg/kg) of guinea-pigs sensitized according to procedure (a) did not result in any depressed antigen-induced bronchial anaphylaxis when tests were performed 1, 3 or 7 days after drug administration. Aminophylline 30 and 60 mg/kg, given as a single injection resulted in an inhibition of the antigen-induced bronchospasm 1 day, but not 3 and 7 days, after treatment. Guinea-pigs sensitized according to procedure (a) and undergoing long-term treatment with 10 or 60 mg/kg aminophylline showed a depressed antigen-induced bronchial anaphylaxis 1 day, but not 3 or 7 days, after treatment ceased. Long-term treatment with 30 mg/kg resulted in an inhibition of the antigen-induced response 1 and 3 days after treatment ceased. However, the response 7 days after the last administration had returned to the control level.

Effect of long-term treatment with sodium cromoglycate and aminophylline on antigen-induced bronchial anaphylaxis in guinea-pigs sensitized to produce only IgG-like antibodies (Procedure b)

The prechallenge level of R_L and C_{Dyn} was 22.7 ± 2.8 cmH_2O l^{-1} s^{-1} and 2.24 ± 0.16 ml/cmH_2O, respectively (mean ± s.e.mean, $n = 24$). Long-term treatment with aminophylline, 30 mg/kg daily, resulted in a reduced response to antigen 3 days after cessation of the drug. However, long-term treatment with SCG, 10 mg/kg daily, did not result in any decreased reactivity 3 days after the last drug administration (Figure 2).

Effect of long-term treatment with sodiumcromoglycate and aminophylline on histamine release in sensitized guinea-pigs

The effect of long-term treatment with SCG (10 mg/kg) and aminophylline (30 mg/kg) on antigen-induced histamine release was investigated with chopped lung technique 3 days after the treatment ceased. The total histamine content was 7.6 ± 1.1 μg/g (mean ± s.e.mean, $n = 26$) in lungs taken from animals sensitized according to procedure (a) and 7.2 ± 0.6 μg/g ($n = 12$, NS) in lungs taken from guinea-pigs sensitized according to procedure (b). From the results summarized in Figure 3 it can be

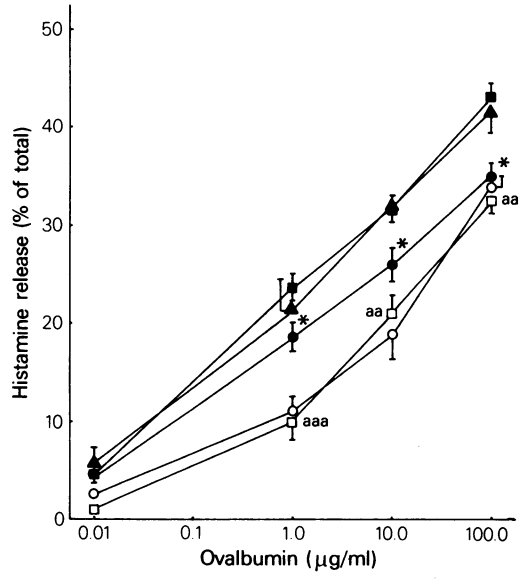

Figure 3 Inhibition of antigen-induced histamine release (mean values, vertical lines show s.e.mean) after long-term treatment with sodium cromoglycate (SCG) or aminophylline in guinea-pigs sensitized by procedure (a) or (b). Guinea-pigs sensitized by procedure (a): (■) control response ($n = 12$); (●) SCG-treated ($n = 8$); (▲) aminophylline-treated ($n = 6$). Guinea-pigs sensitized by procedure (b): (□) control response ($n = 6$); (○) SCG-treated ($n = 6$). *Indicates significance between untreated and long-term treated guinea-pigs *$0.05 > P > 0.01$. [a]Indicates significance between guinea-pigs sensitized by procedures (a) and (b). [aa]$0.01 > P > 0.001$; [aaa]$P < 0.001$.

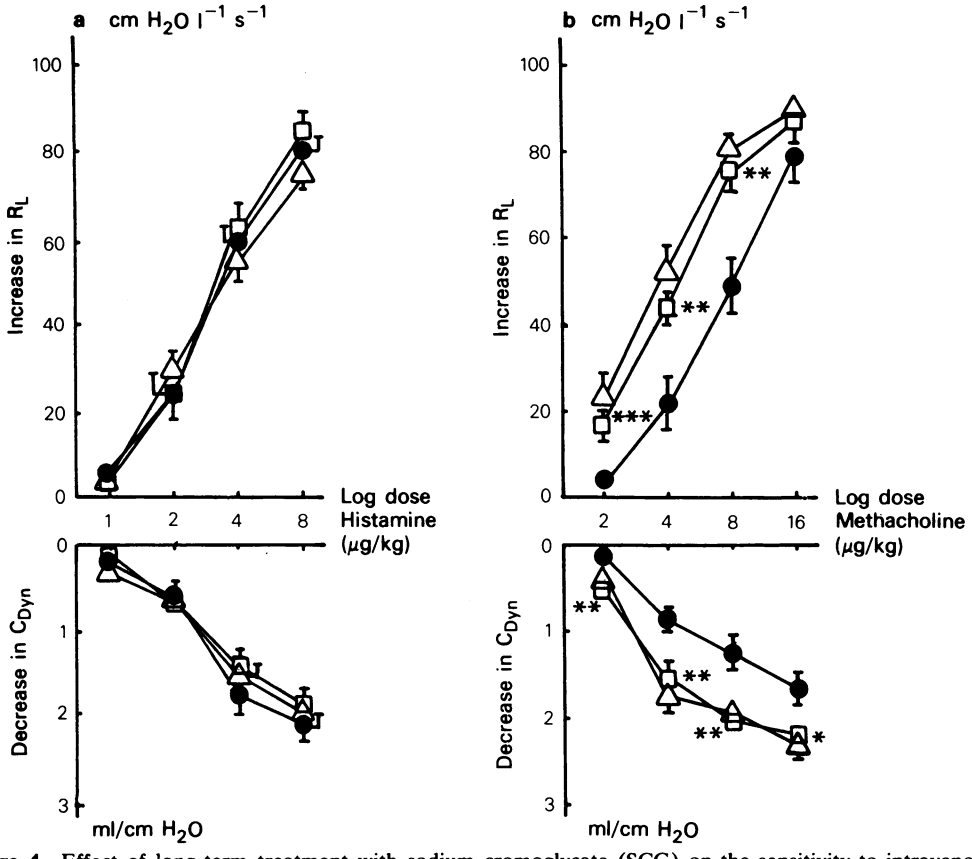

Figure 4 Effect of long-term treatment with sodium cromoglycate (SCG) on the sensitivity to intravenously administered histamine or methacholine in guinea-pigs sensitized by procedure (a). (a) Histamine sensitivity: (●) unsensitized guinea-pigs ($n=8$); (□) sensitized guinea-pigs ($n=8$); (△) sensitized and SCG-treated guinea-pigs ($n=8$). (b) Methacholine sensitivity : (●) unsensitized guinea-pigs ($n=8$); (□) sensitized guinea-pigs ($n=8$); (△) sensitized and SCG-treated guinea-pigs ($n=8$). Points are mean and vertical lines show s.e.mean. $^{**}0.01>P>0.001$; $^{***}P<0.001$.

seen that when challenged with antigen, lungs taken from guinea-pigs sensitized according to procedure (b) released less histamine than those taken from guinea-pigs sensitized according to procedure (a). Histamine release from animals sensitized according to procedure (a) and with long-term treatment with SCG is less than that of the corresponding controls. However, lung tissue from animals sensitized according to procedure (b) did not show decreased histamine release after long-term treatment with SCG. No effect of long-term treatment with aminophylline was found in guinea-pigs sensitized according to procedure (a).

The effect of histamine, methacholine, atropine and isoprenaline after long-term treatment with sodiumcromoglycate or aminophylline in sensitized guinea-pigs

In guinea-pigs sensitized according to procedure (a) there is no difference in sensitivity to intravenously given histamine between unsensitized, sensitized or sensitized SCG-treated guinea-pigs (Figure 4a). When the sensitivity to intravenously given methacholine was examined, a significant increase in methacholine sensitivity was found. However, this increased sensitivity to methacholine was not affected by long-term treatment with SCG (10 mg/kg, daily) (Figure 4). Atropine (1 mg/kg) caused a significant decrease in animals sensitized according to procedure (a). However, guinea-pigs sensitized according to procedure (b) showed no decrease in the antigen-induced bronchial response after atropine treatment (Figure 5). A decreased sensitivity to isoprenaline was found in tracheas taken from guinea-pigs sensitized according to procedure (a): (ED_{50} $2.2\pm0.3\times10^{-3}$ μg/ml, $0.01>P>0.001$, $n=8$) compared with unsensitized animals (ED_{50} $0.8\pm0.2\times10^{-3}$ μg/ml, $n=8$).

As the effect of isoprenaline was compared in

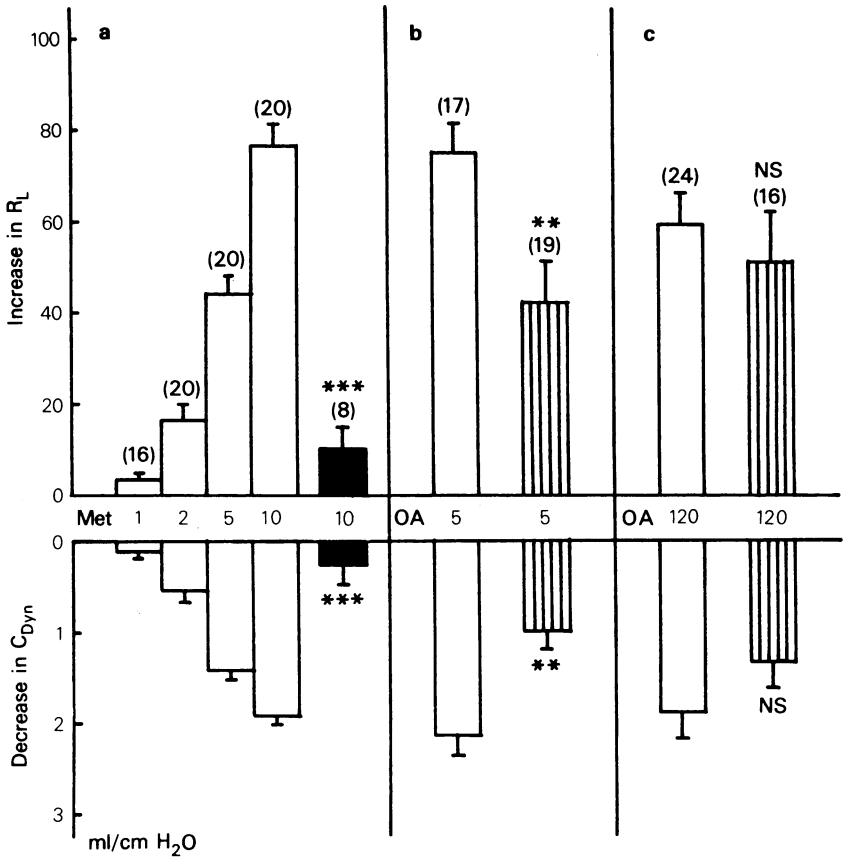

Figure 5 Effect of atropine on an antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Test performed on day 63 after sensitization. Mean values are shown; vertical lines indicate s.e.mean. (a) Dose-response with methacholine, 1, 2, 5 and 10 μg/kg in guinea-pigs sensitized according to procedure (a). Open columns methacholine (1, 2, 5 and 10 μg/kg, i.v.); solid columns atropine (1 mg/kg, i.v.) 10 min before methacholine (10 μg/kg). (b) Guinea-pigs sensitized with ovalbumin (OA) 1 μg (plus) Al $(OH)_3$ 100mg. Provocation dose, ovalbumin 5 μg/kg. Control response, open columns. Atropine (1 mg/kg i.v.) 10 min before ovalbumin (5 μg/kg), striped columns. (c) Guinea-pigs sensitized with ovalbumin 5 mg (day 0) and 10 mg (day 2). Provocation dose, 120 μg/kg. Control response, open columns. Atropine (1 mg/kg i.v.) 10 min before ovalbumin (120 μg/kg), striped columns. Numbers in parentheses indicate the number of experiments. NS = not significant; $***P<0.001$; $**0.01>P>0.001$.

sensitized and sensitized-drug-treated animals there was a decrease in sensitivity in SCG-treated (10 mg/kg, daily) animals (ED_{50} $0.6\pm0.1\times10^{-3}$ μg/ml, $0.01>P>0.001$, $n=8$) but not in aminophylline-treated (30 mg/kg, daily) guinea-pigs (ED_{50} $1.7\pm0.1\times10^{-3}$ μg/ml, mean ± s.e.mean, NS, $n=8$).

Discussion

IgE-antibody mediated reactions play an important role in the pathogenesis of human atopic diseases (Johansson & Foucard, 1978). There are reports indicating involvement of IgG antibodies in the release of anaphylactic mediators in man but their role in the pathogenesis of atopic diseases like asthma is controversial (Bryant, Burns & Lazarus, 1973; 1975). Andersson (1980a; 1981) showed that guinea-pigs sensitized with low doses of antigen together with Al$(OH)_3$ as an adjuvant (procedure a) produce both IgE- and IgG-like antibodies whereas those sensitized with high doses of antigen without adjuvant (procedure b) produce IgG-like antibodies.

There are reports indicating different receptors for IgG and IgE mast cells (Daëron, Pronovost-Danon & Voisin, 1980; Moodley & Morgar 1981). Andersson (1980a) showed that much higher provocation doses must be used to induce a bronchial response in guinea-pigs sensitized according to procedure (b)

compared with those sensitized according to procedure (a). The present results confirm those obtained earlier, and also show that there is a significant difference in degree of histamine release at antigen provocation to lungs taken from guinea-pigs sensitized to produce IgE-like antibodies compared to the release obtained in lungs taken from guinea-pigs sensitized to produce IgG-like antibodies. This is in accordance with the results in mice reported by Barnett & Justus (1975). Moreover, the present investigation shows that acute administration of atropine decreases the antigen-induced bronchial anaphylaxis in guinea-pigs sensitized to produce IgE-like antibodies, but no effect of atropine is seen in those sensitized to produce only IgG-like antibodies. These results indicate that there is a qualitative difference between the two types of antigen-antibody mediated reactions.

Early studies suggested that SCG was active only as a specific inhibitor of IgE-mediated reactions (Altounyan, 1967). Other studies suggest that the acute effects of SCG in extrinsic asthma are due to its ability to stabilize mast cells independently of the stimulus applied and thus prevent the release of mediators (Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard, 1970). Clinical trials with SCG have shown a strong carry-over effect after long-term treatment (Kennedy, 1969; Bernstein, Siegel, Brandon, Brown, Evans, Feinberg, Friedlaender, Krumholz, Hadely, Handelman, Thurson & Yamata, 1972). In the guinea-pig model used in the present experiments, we show an inhibition of the ovalbumin induced bronchial anaphylaxis after long-term treatment with SCG. This is achieved in guinea-pigs sensitized to produce a long-lasting hypersensitive state which is mediated at least partly by IgE-antibodies. Inhibition persisted even seven days after treatment ceased. Qualitatively similar results were found when the degree of antigen-induced histamine release from chopped lung tissue was examined after long-term treatment with SCG or saline. Thus long-term treatment with SCG could conceivably reduce the mediator-releasing potential of lung tissue mast cells.

However, results have recently been presented that throw doubt on the relative importance of mast cell degranulation as the mechanism of action of acute treatment with SCG (Church & Gradidge, 1980) and other mechanisms of action have been considered. Histamine, one of the main mediators liberated at an antigen-antibody reaction in guinea-pigs, is thought to trigger a bronchoconstriction through an 'irritant' receptor, parasympathetic reflex mechanism (for references see Boushey, Holtzman, Sheller & Nadel, 1980). Jackson & Richards (1977), using dogs, demonstrated that SCG inhibits a bronchospasm induced by histamine aerosol but not one induced by electrical stimulation of the vagus nerve. They suggest that SCG reduces the reactivity of the afferent vagal pathway probably by action on the 'irritant' receptors. Our results do not contradict that possibility.

In the pathogenesis of human asthma, hyperreactivity to released mediators is one of the proposed reasons for the severity of the disease (Boushey *et al.*, 1980). One mechanism behind the effect of SCG could be that the compound reverses the changed reactivity to mediators participating in the anaphylactic reaction (Altounyan, 1980). However, there are reports which deny that possibility (for references see Church, 1978).

In the present investigation, long-term treatment with SCG did not change the mediator reactivity. It has been suggested that one reason for the hyperirritability of the airways in asthmatics might be a β-adrenoceptor blockade (for references see Reed & Townley, 1978; Boushey *et al.*, 1980). Interestingly, tracheal tissue from sensitized animals in the present experiments showed decreased responsiveness to isoprenaline. Long-term treatment with SCG was found to increase the reactivity to isoprenaline. These results are in agreement with those obtained by Mahajani & Kulkarni (1977). The decreased reactivity to isoprenaline found in the present investigation could be explained by the increased reactivity to cholinoceptor stimulants. However, since no effect of SCG treatment on the methacholine sensitivity was found, this could not explain the changed isoprenaline reactivity after long-term treatment with SCG.

Theophylline has been shown to exert several actions beneficial in the treatment of bronchial asthma such as relaxation of bronchial smooth muscle, stimulation of mucociliary clearance, suppression of pulmonary oedema and inhibition of mediator release (Persson, 1980; Kaliner & Austen, 1974; Holroyde, Burka & Eyre, 1977).

Andersson (1980b) showed that aminophylline given intravenously inhibited the antigen-induced bronchial anaphylaxis independently of the mode of immunization at a concentration that did not show bronchodilator properties when tested against a histamine-induced contraction. In the present investigation theophylline, unlike SCG, decreased the bronchial anaphylactic reactivity in guinea-pigs independent of the sensitization regimen. Plasma samples obtained three days after the daily treatment with aminophylline 30 mg/kg ceased showed no detectable amount of theophylline (detection limit 0.02 μg/ml) when analysed for unchanged theophylline by liquid chromotagraphy (h.p.l.c.) (Edholm personal communication). At this time, decreased anaphylactic reactivity was still seen. However, the dose-response curve was bell-shaped and the effect

disappeared within 7 days after the treatment ceased. Possibly a decrease in the titre of anaphylactic antibodies induced by theophylline would lead to a decreased mediator release. Such a decrease was reported by Perper, Blancuzzi & Oronsky (1973) in experiments with sensitized mice. In the present investigation, other reasons for the decreased reactivity must be found, since no decrease in mediator release capacity was seen after long-term treatment with aminophylline. According to Collier & James (1967) and Piper, Collier & Vane (1967), the liberation of adrenaline takes part in anaphylaxis of guinea-pigs, weakening to some degree the bronchospastic reaction. Low concentrations of theophylline and β-receptor interfering drugs have been shown to increase relaxation of the tracheobronchial smooth muscle in a synergistic or additive way (Lefcoe, Toogood & Jones, 1975; Triner, Vulliemoz & Verosky 1977; Hanna & Roth 1979). However, the present investigation shows no potentiation of the effect of isoprenaline in isolated tracheae taken from guinea-pigs undergoing long-term treatment with theophylline and further studies are needed to evaluate the mechanism behind the anti-anaphylactic effect of theophylline.

In conclusion, the present investigation shows that long-term treatment with SCG causes a decreased bronchial response to antigen challenge in guinea-pigs sensitized to produce a long-lasting sensitive state mediated, at least partly, by IgE-antibodies. No effect is seen in guinea-pigs sensitized to produce only IgG-antibodies. These effects agree with those seen on histamine release. The effect of SCG-treatment on β-adrenoceptor reactivity might contribute to the decreased anaphylactic reactivity. Long-term aminophylline treatment produces effects similar to those obtained with SCG. The effect of aminophylline is observed irrespective of the kind of anaphylactic antibody being produced and is not due to inhibition of histamine release or increased β-adrenoceptor activity.

The skilful technical assistance of Mrs G. Stahre and Miss C. Brange and the secreterial work of Mrs I. Källén and Mrs U. Tapper are gratefully acknowledged. We sincerely thank Fisons Ltd for the gift of SCG.

References

ALTOUNYAN, R.E.C. (1967). Inhibition of experimental asthma by a new compound – Disodium cromoglycate, "Intal". *Acta Allerg.*, **22,** 487.

ALTOUNYAN, R.E.C. (1980). Review of clinical activity and mode of action of sodium cromoglycate. *Clin. Allergy*, **10,** Suppl., 481–489.

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. (1981). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. The effect of booster injection and cyclophosphamide treatment. *Int. Archs Allergy appl. Immunol.*, **64,** 249–258.

BARNETT, J.B. & JUSTUS, D.E. (1975). Differential effects of homocytotropic antibodies on the mast cell and anaphylactic responses in actively and passively sensitized mice. *Infection and Immunity*, **11,** 1342–1351.

BERNSTEIN, I.L., SIEGEL, S.C., BRANDON, M.L., BROWN, E.B., EVANS, R.R., FEINBERG, A.R., FRIEDLAENDER, S., KRUMHOLZ, R.A., HADELY, R.A., HANDELMAN, N.I., THURSTON, D., YAMATE, M. (1972). A controlled study of cromolyn sodium sponsored by the drug committee of the American academy of allergy. *J. Allergy clin. immunol.*, **50,** 235–245.

BOUSHEY, H.A., HOLTZMAN, M.J., SHELLER, J.R. & NADEL, J.A. (1980). Bronchial hyperreactivity. *Am. Rev. Resp. Disease*, **121,** 424–428.

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.

CHURCH, M.K. (1978). Cromoglycate-like anti-allergic drugs: A review. Med. Act./Drugs of today, **14,** 281–341.

CHURCH, M.K. & GRADIDGE, C.F. (1980). The activity of sodium cromoglycate analogues in human lung *in vitro*: A comparison with rat passive cutaneous anaphylaxis and clinical efficacy. *Br. J. Pharmac.*, **70,** 307–311.

COLLIER, H.O.J. & JAMES, G.W.L. (1967). Humoral factors affecting pulmonary inflation during acute anaphylaxis in the guinea-pigs in vivo. *Br. J. Pharmac. Chemother.*, **30,** 283–301.

COX, J.S.G., BEACH, J.E., BLAIR, A.M.J.N., CLARKE, A.J., KING, J., LEE, T.B., LOVEDAY, T.E.E., MOSS, G.F., ORR, T.S., RITCHIE, J.T. & SHEARD, P. (1970). Disodium cromoglycate (Intal). *Adv. Drug. Res.*, **5,** 115–193.

DAËRON, M., PRONOVOST-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.

FINNEY, D.J. (1952). *Statistical Methods in Biological Assay*, London: Charles Griffin & Co. Ltd.

HANNA, C.J. & ROTH, S.H. (1979). Combination bronchodilators: antagonism of airway smooth muscle contractions in vitro. *Agents & Actions*, **9**, 18–23.

HARRIES, M.G., PARKES, P.E.G., LESSOF, M.H. & ORR, T.S.G. (1981). Role of bronchial irritant receptors in asthma. *Lancet*, **i**, 5–7.

HOLROYDE, M.C., BURKA, J.F. & EYRE, P., (1977). Automodulation of release of pharmacological mediators of immediate (Type I) hypersensitivity. A Review. *Agents & Actions*, **7**, 421–430.

JACKSON, D.M. & RICHARDS, I.M. (1977). The effects of sodium cromoglycate on histamine aerosol-induced reflex bronchoconstriction in the anaesthetized dog. *Br. J. Pharmac.*, **61**, 257–262.

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

KALINER, M. & AUSTEN, K.F. (1974). Hormonal control of the immunologic release of histamine and slow-contracting substance of anaphylaxis from human lung. In *Cyclic AMP, Cell Growth and the Immune Responses.* eds. Braun, W., Lichtenstein, L.M. & Parker, C.W. pp. 163–176. New York: Springer-Verlag.

KENNEDY, M.C.S. (1969). Disodium cromoglycate in the control of asthma. A double-blind trial. *Br. J. Dis. Chest.*, **63**, 96–106.

LEFCOE, N.M., TOOGOOD, J.H. & JONES, T.R. (1975). In vitro pharmacologic studies of bronchodilator compounds: interactions and mechanisms. *J. Allergy. Clin. Immunol.*, **55**, 94.

LICHTENSTEIN, L.M. & MARGOLIS, S. (1968). Histamine release in vitro: Inhibition by catecholamines and methylxanthines. *Science*, **161**, 902–903.

MAHAJANI, S.S. & KULKARNI, R.D. (1977). Effect of pre-treatment with disodium cromoglycate and pictorrhiza kurroa root powder on sensitivity of guinea-pigs to histamine and sympathomimetic amines. A correlation with their anti-anaphylactic effects. *Int. Archs Allergy appl. Immunol.*, **53**, 137–144.

MAY, C.D., LYMAN, M., ALBERTO, R. & CHENG, J. (1970). Procedures for immunochemical study of histamine release from leucocytes with small volume of blood. *J. Allergy*, **46**, 12–20.

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

ORANGE, R.P., AUSTEN, W.G. & AUSTEN, K.F. (1971). Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3′, 5′-adenosine monophosphate. *J. exp. Med.*, **134**, 136S–148S.

PERPER, R.J., BLANCUZZI, V. & ORONSKY, A.L. (1973). The selective inhibition of secondary IgE antibody response in mice by dibutyryl adenosine 3′,5′-cyclic monophosphoric acid and theophylline. *Proc. Soc. exp. Biol. Med.*, **143**, 1191–1196.

PERSSON, C.G.A. (1980). Some pharmacological aspects on xanthines in asthma. *Eur. J. Respir. Dis.*, Suppl. 109, **61**, 7–16,

PIPER, P.J., COLLIER, H.O.J. & VANE, J.R. (1967). Release of catecholamines in the guinea-pig by substances involved in anaphylaxis. *Nature, Lond.*, **213**, 838–840.

REED, C.E. & TOWNLEY, R.G. (1978). Asthma, classification and pathogenesis. pp. 659–677. In *Allergy, Principles and Practice.* eds. Middleton Jr., E., Reed, C.E. & Ellis, E.F. St. Louis, U.S.A.: C.V. Mosby Co.

TRINER, L., VULLIEMOZ, Y. & VEROSKY, M. (1977). Cyclic 3′,5′-adenosine monophosphate and bronchial tone. *Eur. J. Pharmac.*, **41**, 37–46.

(*Received April 6, 1981.*
Revised June 25, 1981.)