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A COMPARISON OF THE ANTI-ANAPHYLACTIC ACTIVITIES OF SALBUTAMOL AND DISODIUM CROMOGLYCATE IN THE RAT, THE RAT MAST CELL AND IN HUMAN LUNG TISSUE

P.R. BUTCHERS, [1]J.R. FULLARTON, I.F. SKIDMORE, L.E. THOMPSON, C.J. VARDEY & A. WHEELDON

Department of Biochemistry, Glaxo-Allenburys Research Limited, Ware, Hertfordshire, SG12 0DJ

1 Salbutamol and disodium cromoglycate were compared for anti-anaphylactic activity against passive anaphylaxis in rat skin and peritoneum *in vivo* and in rat mast cells and human lung fragments *in vitro*.

2 Salbutamol administered intravenously to rats inhibited cutaneous anaphylaxis, but also inhibited cutaneous responses to histamine and 5-hydroxytryptamine. Salbutamol administered intraperitoneally inhibited the release of slow reacting substance of anaphylaxis (SRS-A) but not the release of histamine in the peritoneum. It was a very weak inhibitor of histamine release from rat mast cells *in vitro*.

3 Disodium cromoglycate administered intravenously to rats inhibited cutaneous anaphylaxis. Disodium cromoglycate administered intraperitoneally to rats inhibited the release of histamine and, to a lesser extent, SRS-A in the peritoneum. It was an effective but short-acting inhibitor of histamine release from rat mast cells *in vitro*.

4 Salbutamol was a potent inhibitor of the anaphylactic release of histamine and SRS-A from fragments of human lung.

5 Disodium cromoglycate was a weak inhibitor of the anaphylactic release of histamine and SRS-A from fragments of human lung. The inhibition was variable and not dose-related.

6 The concentration of salbutamol required to inhibit anaphylaxis in human lung is of the same order as that required to relax human bronchial muscle. It is suggested that salbutamol may be more effective in allergic asthma if given in a prophylactic regimen.

Introduction

In an allergic asthmatic reaction, allergen reacts with immunoglobulin E on the surface of the mast cell to initiate a series of biochemical events resulting in the release of the pharmacologically active substances responsible for the symptoms of the disease. The response is biphasic. An immediate pathopharmacological constriction of the airway is followed by a more slowly developing and persistent inflammatory reaction (Austen & Orange, 1975).

The primary mediators of the response originate from the mast cell itself. Of these primary mediators, histamine and slow reacting substance of anaphylaxis (SRS-A) constrict smooth muscle directly (Collier, 1970) and also, in the case of histamine, indirectly via irritant-sensitive receptors and a vagal reflex (Nadel, 1973). They also increase vascular permeability and can cause oedema (Orange, Stechschulte & Austen, 1969). Anaphylaxis also releases chemotactic factors for eosinophils (ECF-A) (Kay & Austen 1971) and neutrophils (NCF-A) (Lewis, Goetzl, Wasserman, Valone, Rubin & Austen, 1975) which stimulate the accumulation of these cells at the site of reaction and promote the inflammatory response.

In addition, kinins and prostaglandins may be generated either as primary mediators or as secondary mediators resulting from the release of primary mediators and their interaction with cells, tissues and plasma proteins (Piper & Walker, 1973; Austen & Orange, 1975; Strandberg, Mathé & Yen, 1977). Both the kinins and the prostaglandins are vasoactive (Elliot, Horton & Lewis, 1960; Kaley & Weiner, 1971) and can constrict bronchial smooth muscle (Collier, 1970; Smith & Cuthbert, 1972) while the prosta-

[1] Present address: Corning Medical, Corning Ltd., Halstead, Essex.

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glandins also potentiate the effects of both kinins and histamine on vascular permeability (Moncada, Ferreira & Vane, 1973; Williams & Morley, 1973).

The primary and secondary mediators of the anaphylactic response therefore have the properties required to establish both a rapid bronchoconstriction and an inflammatory reaction. The dependence of both bronchoconstriction and inflammatory responses on the initial anaphylactic reaction suggests that drugs which prevent the release of mediators have the potential to be more valuable in the treatment of asthma than drugs that solely reverse the bronchoconstriction that follows the release of mediators.

It is now well established that compounds such as β-adrenoceptor agonists and certain prostaglandins, which activate adenylate cyclase, inhibit the release of histamine, SRS-A and ECF-A from sensitized fragments of human lung challenged with antigen (Austen, 1974). The β_2-adrenoceptor agonist, salbutamol, shares these properties (Assem & Schild, 1971). Disodium cromoglycate (DSCG), an established drug for the treatment of allergic asthma also inhibits the release of histamine and SRS-A from human lung (Sheard & Blair, 1970). The purpose of the present work was to compare directly the effects of salbutamol and DSCG on a range of anaphylactic reactions *in vivo* and *in vitro* in the rat, and *in vitro* on human lung tissue, and to establish whether there is an experimental basis for the prophylactic use of salbutamol in allergic asthma.

Methods

Production of antiserum in the rat

Male hooded rats (250 g) were injected intramuscularly with 10 mg dinitrophenylated ovalbumin (DNP-Ea) and simultaneously were given an intraperitoneal injection of 3×10^{10} *Bordetella pertussis* organisms (Burroughs-Wellcome). After 13 days the animals were anaesthetized with halothane/nitrous oxide and bled from the aorta. The blood was allowed to clot and the serum stored in aliquots at −20°C.

Human reaginic serum

Human reaginic serum was obtained from a pollen sensitive donor (A.W.) and stored in aliquots at −20°C.

Rat passive cutaneous anaphylaxis (Rat PCA)

Female albino rats (100 to 200 g) were shaved on the back and sides and injected intradermally (i.d.) in two sites on the back with 0.1 ml of DNP-ovalbumin anti-serum diluted in saline (the dilution was calculated on the basis of preliminary experiments to give an intense blue weal, 1 to 1.5 cm diameter, on intravenous challenge with antigen and Evans blue). After 48 h the animals were challenged intravenously with 2 mg DNP-Ea in 0.5 ml 1% w/v Evans blue in isotonic saline. Drugs (0.1 ml aqueous solution) were injected intravenously with the antigen; control animals were given 0.1 ml of the drug vehicle. After 30 min the animals were killed by cervical dislocation and the dorsal skin reflected. The size and intensity of the weal was assessed visually. The effect of drugs was scored on a five point scale. (Absence of weals = 100% inhibition; faint, incomplete weals, 0.3 to 0.5 cm diameter = 75% inhibition; pale complete weals, 0.5 to 1.0 cm diameter = 50% inhibition; dark weals, 1.0 to 1.5 cm diameter = 25% inhibition; dark weals $\geqslant$ 1.5 cm diameter = no inhibition.)

Animals given drug vehicle instead of drug always produced very dark large weals. Each dose of a compound was evaluated in 4 rats for the ability to reduce the size and intensity of the weals.

Weals caused by histamine and 5-hydroxytryptamine

In experiments where the inhibitory effects of a drug on a PCA reaction and on cutaneous responses to histamine and 5-hydroxytryptamine were compared, sensitized animals were injected intradermally at separate sites with 5 μg histamine (in 0.1 ml saline) and 0.3 μg 5-hydroxytryptamine (in 0.1 ml saline) immediately before intravenous challenge with antigen, drug or vehicle, and Evans blue as described in the previous section. The intensity of the reaction was assessed as for the rat PCA.

Rat passive peritoneal anaphylaxis (Rat PPA)

Antiserum to DNP-Ea (2 ml of a 1:4 dilution in bicarbonate buffered Tyrode solution containing (mM) NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, and D-glucose 5.6, pH 7.4) was injected into the peritoneal cavity of each of a group of 4 female albino rats (125 to 180 g). After 2 h the animals were challenged intraperitoneally with 2 mg DNP-Ea in 5 ml of bicarbonate-buffered Tyrode containing 250 μg heparin. Drug or drug vehicle (0.75 ml) was injected immediately prior to challenge. Five minutes after challenge the animals were killed by cervical dislocation and the peritoneal fluid removed. The fluid was centrifuged at 200 *g* for 5 min to separate the cells and the supernatant fluid was assayed for histamine and for SRS-A. Each dose of compound was evaluated in 4 rats and the mean percentage inhibition of histamine and SRS-A release calculated.

Anaphylaxis in suspended rat peritoneal and pleural mast cells

Albino male rats (150 to 250 g) were decapitated and exsanguinated. Tris buffered medium (pH 7.6), containing disodium edetate (EDTA) 0.1 mM, NaCl 123 mM, KCl 2.7 mM, Tris 25 mM, D-glucose 5.6 mM and gelatin (0.1% w/v) (Tris/EDTA) was then injected, 6 ml into the peritoneal and 4 ml into the pleural cavities. The body was gently massaged for about 30 s and the peritoneal and pleural fluids removed. Any fluid contaminated with blood was discarded. The fluid from several rats was pooled and centrifuged at 200 *g* for 5 min. The supernatant was discarded and the pellet of cells washed three times in Tris buffered medium as above but lacking the EDTA (EDTA-deficient Tris buffer). After the third wash the cells were resuspended in EDTA-deficient Tris buffer to give a mast cell concentration between 2.5 to 3.0×10^5 cells/ml. (Mast cells, which comprise about 5% of total cells, were counted in a haemocytometer after staining with methylene blue.)

One ml of rat DNP-Ea reaginic anti-serum was added to each 10 ml of cell suspension and the cells incubated for 2 h at 37°C. The suspension was then centrifuged at 200 *g* for 5 min, the supernatant discarded and the cells washed twice in EDTA-deficient Tris buffer as before. After the final wash the cells were suspended in Tris buffered medium, pH 7.6 containing NaCl 123 mM, KCl 2.7 mM, $CaCl_2$ 1.0 mM, $MgCl_2$ 1.0 mM, Tris 25 mM, D-glucose 5.6 mM and 0.1% (w/v) gelatin (Tris/Ca^{2+}/Mg^{2+} buffer) to give a mast cell concentration of between 1.2 and 1.7×10^5 cells/ml. Aliquots (500 µl) of the final cell suspension were pipetted into 2 ml plastic tubes and incubated at 37°C for 15 min. Then 50 µl of antigen solution, diluted in Tris/Ca^{2+}/Mg^{2+} buffer, was added to each 500 µl of cell suspension at 37°C. The optimum concentration of antigen in the final suspension was calculated to release approximately 30% of total cell histamine and varied from 3 ng/ml to 500 ng/ml DNP-Ea, depending on the potency of the anti-serum. Each incubation was performed in duplicate. To measure non-anaphylactic release of histamine 50 µl of the Tris/Ca^{2+}/Mg^{2+} buffer instead of antigen solution was added to 500 µl of cell suspension.

After challenge the cells were incubated for 15 min at 37°C and then centrifuged as before. The supernatant was assayed for released histamine. Distilled water (1 ml) was added to the residual cell pellet and the lysed cells were frozen overnight at −20°C. After thawing the resultant solution was assayed for residual histamine. Histamine release was expressed as a percentage of the total histamine content of the cells, i.e. released plus residual histamine and corrected for non-anaphylactic histamine release by subtraction. Drugs were dissolved in water and 10 µl of the solution were added to 500 µl cell suspension simultaneously with, or at various times before, the antigen; 10 µl of water was added to drug-free control reactions.

Each drug effect was expressed as:

$$\%\ \text{inhibition} = \frac{\left(\begin{matrix}\%\ \text{histamine released} \\ \text{in drug-free controls}\end{matrix}\right) - \left(\begin{matrix}\%\ \text{histamine released in} \\ \text{drug-treated sample}\end{matrix}\right)}{\%\ \text{histamine released in drug-free controls}} \times 100$$

Anaphylaxis in fragments of human lung in vitro

Normal parenchyma dissected from lung tissue obtained from patients undergoing surgical removal of carcinomas of the lung and bronchi was washed free of blood with bicarbonate-buffered Tyrode solution.

The tissue was cut into small (2 to 3 mm^3) pieces and these fragments of lung were sensitized passively overnight at room temperature in a 1:5 or 1:10 dilution of reaginic serum. The tissue fragments were washed thoroughly in bicarbonate-buffered Tyrode solution to remove unbound components of the serum, and distributed into plastic tubes containing 500 µl Tyrode solution. The fragments were incubated for 15 min at 37°C and then challenged with 50 µl of antigen solution (dialysed B_2 grass pollen—Bencard) at an experimentally determined optimum concentration (1 to 20 µg protein/ml) calculated to release not more than 40% of the total histamine. Non-anaphylactic release was determined by challenge with 50 µl of Tyrode solution.

Aqueous solutions of drug (10 µl), or water (10 µl), were added at various times before challenge with antigen. Fifteen minutes after challenge the supernatants were removed for bioassay of released histamine and SRS-A. The remaining fragments of tissue were boiled in 1.0 ml distilled water for 5 min and the resulting suspensions assayed for residual histamine. The release of histamine and the effects of drugs on this process were calculated as for the rat mast cell.

The SRS-A released during anaphylaxis is not preformed but synthesized following challenge. It is not possible, therefore, to express SRS-A release as a function of total tissue SRS-A. Accordingly, to correct for variation in the sizes of lung fragments, SRS-A release was related to the total histamine content of the lung fragments and expressed as SRS-A units released per unit total tissue histamine.

Assay of histamine

Histamine was assayed on the isolated guinea-pig ileum by an automated superfusion technique. The Tyrode superfusion solution contained atropine 1.44 μM. Histamine samples of unknown concentration were bracketed by high and low standards. From the peak height of the unknown and its pair of bracketing standards the histamine content of the unknown sample was calculated.

Assay of SRS-A

SRS-A was assayed on the guinea-pig isolated ileum by an automated superfusion technique. The Tyrode solution contained atropine 1.44 μM and mepyramine 2.5 μM. Arbitrary standards of SRS-A were prepared for each experiment and a standard concentration curve obtained before, during and after the assay. Unknown samples were assayed by comparison with these standard solutions.

Drugs

All drug solutions were made up immediately before use. Concentrations of disodium cromoglycate refer to the disodium salt, concentrations of (±)-salbutamol refer to the free base. Histamine and 5-hydroxytryptamine doses refer to the free base.

Statistical treatment of results

Where dose-response relationships were sigmoid the method of O'Neill (1971) was used to provide EC_{50} values, otherwise a straight line relationship was fitted between response and logarithm of dose or concentration. The 95% confidence limits were derived from the variation of estimates in replicate experiments. When only a single experiment was carried out approximate limits were computed from the internal variation.

Results

Inhibition of passive cutaneous anaphylaxis in the rat
Intravenous salbutamol (1 to 25 μg/kg) given with the antigen caused a dose-dependent inhibition of the PCA reaction in the rat, the ED_{50} being 3.4 (1.6 to 5.8) μg/kg. Doses of salbutamol which inhibited the PCA reaction effectively (5 to 25 μg/kg, 50 to 85% inhibition) also reduced weals caused by intradermal injection of histamine and 5-hydroxytryptamine (15 to 40% inhibition). Intravenous DSCG (0.5 to 5 mg/kg) given with the antigen caused a dose-dependent inhibition of the PCA reaction, with an ED_{50} of 1.25 (1.10 to 1.40) mg/kg. Intravenous DSCG (5 mg/kg) did not inhibit the cutaneous response to histamine or 5-hydroxytryptamine.

Inhibition of passive peritoneal anaphylaxis in the rat (Table 1)

Intraperitoneal salbutamol given with the antigen inhibited the anaphylactic release of SRS-A into the peritoneal cavity (ED_{50} 0.44 (0.36 to 0.51) μg/kg) but intraperitoneal salbutamol up to 2 μg/kg did not inhibit the release of histamine. Intraperitoneal DSCG given with the antigen inhibited the release of histamine (ED_{50} 70 (59 to 81) μg/kg) and to a lesser extent of SRS-A ($ED_{50} > 640$ μg/kg).

Inhibition of the anaphylactic release of histamine from rat mast cells in vitro

Salbutamol was a poor inhibitor of histamine release from pleural and peritoneal mast cells of the rat; 90 μg/ml of the drug caused less than 40% inhibition of release even after a 20 min pre-incubation period and lower concentrations were even less effective. DSCG (0.18 to 18 μg/ml) added to the mast cells with the antigen caused a concentration-dependent inhibition of the release of histamine, EC_{50} being 4.8 (3.4 to 6.3) μg/ml. Preincubation of DSCG (90 μg/ml) with the cells for 5 min before the addition of antigen reduced the inhibition of histamine release from 56 to 3%. Pretreatment of the mast cells with DSCG (45 μg/ml) for 30 min in the absence of antigen desensitized the cells to a second addition of DSCG at the same time as the antigen (Table 2).

Inhibition of anaphylaxis in fragments of human lung

Salbutamol (0.48 to 60 ng/ml) caused a concentration-dependent inhibition of the anaphylactic release of histamine from human lung (Table 3). Preincubation (up to 20 min) of the lung fragments with salbutamol marginally increased the potency of the drug; the EC_{50} was 11.9 (11.1 to 12.7) ng/ml, when given simultaneously with antigen and 5.2 (2.6 to 7.8) ng/ml when given 20 min before antigen.

DSCG (0.18 to 180 μg/ml) was poorly effective in preventing the release of histamine at all preincubation times (Table 3). Potentiation of release was observed at some concentrations. Maximal activity was seen when the compound was added simultaneously with antigen; the EC_{50} was 7.8 μg/ml (no limits).

In a series of experiments comparing the activities of salbutamol with DSCG, compounds were added 10 min before antigen (Table 4), or simultaneously with antigen (Tables 5 and 6), and both histamine and SRS-A release were measured. Salbutamol (2 to 162 ng/ml) caused a concentration-dependent inhibi-

tion of the release of histamine; the EC_{50} was 17.8 (1.2 to 260) ng/ml when given 10 min before antigen (Table 4) and 38 (15 to 96) ng/ml when given simultaneously with antigen (Table 5). In contrast to salbutamol, DSCG was found to be a weak inhibitor of the release of histamine (Tables 4 and 5). Results were very variable between experiments, and within experiments, responses were frequently not dose-related. Even at high concentrations (900 μg/ml) inhibition rarely exceeded 50%. The release of SRS-A was more sensitive than histamine release to inhibition by salbutamol both with a 10 min preincubation (EC_{50} 3.5 (1.7 to 7.1) ng/ml, Table 4), and with no preincubation (EC_{50} 5.2 (1.3 to 20.8) ng/ml, Table 6). Similarly, DSCG although much less potent than salbutamol, was a more effective inhibitor of SRS-A release than of histamine release but only when given simultaneously with the antigen. Here, the inhibition was apparently biphasic and no single EC_{50} could be calculated from statistical treatment of the data (Table 6). The response to DSCG showed a peak at 1.44 μg/ml and 180 μg/ml.

Discussion

The response of the sensitized mast cell to antigen is probably the key event in the allergic reactions characterizing extrinsic asthma, hay fever and urticaria. It is therefore a major target in the treatment

Table 1 Inhibition of passive peritoneal anaphylaxis in the rat by salbutamol and disodium cromoglycate (DSCG) given intraperitoneally with the antigen

Salbutamol μg/kg i.p. (3 *animals per group*)	0.05	0.10	0.25	0.5	1.0	2.0
% inhibition of histamine release (mean ± s.e. mean)	2 ± 21	2 ± 15	−6 ± 20↑	1 ± 17	10 ± 15	−10 ± 7↑
% inhibition of SRS-A release (mean ± s.e.mean)	38 ± 22	13 ± 34	10 ± 21	63 ± 2	74 ± 3	100 ± 0
ED_{50} 0.44 (0.36–0.51) μg/kg						
Disodium cromoglycate μg/kg i.p. (4 *animals per group*)	10	40	160	640		
% inhibition of histamine release (mean ± s.e.mean)	25 ± 8	33 ± 7	78 ± 3	86 ± 2		
ED_{50} 70 (59–81) μg/kg i.p.						
% inhibition of SRS-A release (mean ± s.e.mean)	20 ± 10	−2 ± 10↑	31 ± 5	42 ± 12		
ED_{50} >640 μg/kg i.p.						

Approximate 95% confidence limits given in parentheses.
↑ Increase in release.

Table 2 Effect of preincubation with one dose of disodium cromoglycate on the ability of a second dose to inhibit histamine release from rat mast cells (*in vitro*)

Addition 30 min before antigen	*Addition with antigen*	*% inhibition (mean ± s.e.mean)*
Water	Disodium cromoglycate (45 μg/ml)	89 ± 0
Disodium cromoglycate (45 μg/ml)	Water	33 ± 0
Disodium cromoglycate (45 μg/ml)	Disodium cromoglycate (45 μg/ml)	26 ± 1

Mast cells were incubated at 37°C for 30 min with the additions in the first column. The second drug treatment was added with the antigen and, after 15 min incubation, histamine release was measured. Results are the means of duplicate incubations.

Table 3 Inhibition of anaphylactic release of histamine from fragments of human lung by salbutamol and disodium cromoglycate: effect of preincubation

	% inhibition of histamine release				
Salbutamol (ng/ml)	0.48	2.4	12	60	EC_{50} (ng/ml)
Preincubation period (min)					
20	−26.9 ± 13.0↑	28.4 ± 16.6	69.0 ± 4.2	94.8 ± 2.8	5.2 (2.6–7.8)
10	1.1 ± 14.8	−2.6 ± 2.8↑	66.4 ± 6.2	101.9 ± 3.7	11.2 (10.7–11.6)
5	−23.2 ± 20.0↑	11.2 ± 19.7	57.0 ± 8.8	90.5 ± 2.7	9.3 (3.3–15.3)
0	9.4 ± 7.8	−2.8 ± 22.7↑	51.6 ± 6.5	94.8 ± 1.6	11.9 (11.1–12.7)
	% inhibition of histamine release				
Disodium cromoglycate (μg/ml)	0.18	1.8	18	180	EC_{50} (μg/ml)
Preincubation period (min)					
20	−32.6 ± 16.0↑	32.8 ± 5.5	39.5 ± 14.5	40.0 ± 2.9	>180
10	−44.5 ± 3.5↑	28.7 ± 14.4	18.2 ± 10.5	12.7 ± 10.0	>180
5	13.1 ± 7.1	30.5 ± 6.0	32.8 ± 7.9	22.8 ± 11.5	>180
0	11.4 ± 19.0	33.0 ± 5.9	54.2 ± 4.6	56.8 ± 7.2	7.8 (No limits)

Mean histamine release of control 8.33 ± 0.56.
Values are means ± s.e.mean of triplicate determinations. Approximate 95% confidence limits given in parentheses.
↑ Increase in histamine release.

Table 4 Inhibition of anaphylactic release of histamine and slow reacting substance of anaphylaxis (SRS-A) from fragments of human lung (10 min preincubation)

Salbutamol ng/ml	0.22	0.66	2	6	18	54
% inhibition of histamine release	−5.3 ± 4.2↑	−9.1 ± 4.5↑	12.6 ± 8.3	6.4 ± 6.4	63.9 ± 16.1	86.0 ± 17.1
EC_{50} 17.8 (1.2–260) ng/ml						
% inhibition of SRS-A release	3.3 ± 7.1	13.8 ± 4.6	35.8 ± 6.8	47.4 ± 12.0	70.6 ± 7.4	80.6 ± 8.2
EC_{50} 3.5 (1.7–7.1) ng/ml						
EC_{50} histamine/EC_{50} SRS-A ratio = 5.1 (1.2–22.7)						
This is sigificantly different from unity, $P < 0.05$						
Disodium cromoglycate μg/ml	1.4	7.2	36	180	900	
% inhibition of histamine release	20.7 ± 5.7	23.6 ± 11.6	17.5 ± 4.9	2.9 ± 9.0	17.1 ± 6.2	
Response not concentration-dependent						
% inhibition of SRS-A release	29.0 ± 10.8	24.2 ± 13.9	13.8 ± 15.6	17.8 ± 15.6	21.0 ± 16.2	
Response not concentration-dependent						

Mean histamine release 37.8 ± 9.1% range 6.8–55.8%.
Values are means ± s.e.mean of five separate experiments performed in duplicate or triplicate. 95% confidence limits are given in parentheses.
↑ Increase in histamine release.

Table 5 Inhibition of anaphylactic release of histamine from fragments of human lung (no preincubation)

Salbutamol ng/ml	0.66	2	6	18	54	162	
% inhibition of histamine release	−11.2 ± 4.5↑	−3.1 ± 6.8↑	10.0 ± 5.3	32.8 ± 6.7	53.8 ± 5.0	67.6 ± 4.5	
EC_{50} 38 (15–96) ng/ml							
Disodium cromoglycate μg/ml	0.058	0.29	1.44	7.2	36	180	900
% inhibition of histamine release	3.3 ± 5.9	7.6 ± 5.4	17.9 ± 14.3	25.1 ± 6.8	26.5 ± 6.3	43.2 ± 5.3	28.4 ± 6.2
No EC_{50}							

Mean histamine release 31.4 ± 6.2, range 16.9–59.3.
Values are means ± s.e.mean of six separate experiments performed in triplicate. 95% confidence limits are given in parentheses.
↑ Increase in histamine release.

Table 6 Inhibition of anaphylactic release of histamine and slow reacting substance of anaphylaxis (SRS-A) from fragments of human lung (no preincubation)

Salbutamol ng/ml	0.66	2	6	18	54	162	486
% inhibition of histamine release	−1.9 ± 5.1↑	10.8 ± 6.9	22.8 ± 5.0	41.6 ± 7.2	53.5 ± 7.7	57.8 ± 9.8	77.0 ± 3.1
EC_{50} 31 (25–37) ng/ml							
% inhibition of SRS-A release	10.6 ± 16.2	−4.8 ± 16.0↑	65.3 ± 1.4	81.8 ± 4.4	90.5 ± 1.4	98.3 ± 2.2	92.0 ± 1.1
EC_{50} 5.2 (1.3–20.8) ng/ml							
EC_{50} histamine/EC_{50} SRS-A ratio 6.0 (1.2–28.5). This is significantly different from unity, $P < 0.05$							
Disodium cromoglycate μg/ml	0.058	0.29	1.44	7.2	36	180	900
% inhibition of histamine release	−11.5 ± 5.4↑	8.9 ± 6.8	19.1 ± 6.3	11.7 ± 10.9	16.5 ± 12.0	40.1 ± 9.4	36.0 ± 5.7
EC_{50} > 900 μg/ml							
% inhibition of SRS-A release	−14.6 ± 23.6↑	34.7 ± 6.1	60.1 ± 4.8	18.3 ± 30.8	30.5 ± 11.3	61.5 ± 4.4	41.5 ± 10.5
No EC_{50} (response biphasic)							

Mean histamine release 40.4% ± 19.0, range 21.4–59.3.
Values are means ± s.e.mean of two separate experiments performed in triplicate. 95% confidence limits given in parentheses.
↑ Increase in histamine release or SRS-A release.

of these diseases. In this paper we compare the effects of two drugs, DSCG an established prophylactic treatment for allergic asthma and hay fever and salbutamol a potent β_2-adrenoceptor agonist, in two of the most widely used 'models' of the anaphylactic response, viz. the anaphylactic reactions of the rat and its mast cells and the release of histamine and SRS-A from fragments of human lung. The results show that the antianaphylactic properties of salbutamol and DSCG are radically different.

Salbutamol is a poor inhibitor of histamine release from rat mast cells both *in vitro*, confirming the observations of Martin (1971) and of Sullivan, Parker, Eisen and Parker (1975) for isoprenaline, and *in vivo*. Some of its activity in the PCA reaction may be due to pharmacological activities on the vasculature that do not involve the mast cell. The poor activity of salbutamol and other β-stimulants against the rat mast cell probably results from the absence of an adequate β-receptor control system for the adenylate cyclase of these cells. Salbutamol does inhibit the release of SRS-A in the PPA reaction of the rat but in this species SRS-A is thought to originate from cells other than the mast cell, such as the neutrophil polymorph (Orange & Austen, 1969) and the monocyte (Bach & Brashler, 1978).

In contrast, DSCG is an efficient short acting inhibitor of histamine release from rat mast cells, an effective inhibitor of the rat PCA reaction and of the release of histamine and to a lesser extent SRS-A in the peritoneal cavity of the rat. The potency and kinetics of inhibition presented here agree closely with published studies (Cox, 1971; Thomson & Evans, 1973; Fullarton, Martin & Vardey, 1973; Kusner, Dubnick & Herzig, 1973).

In fragments of human lung sensitized to and challenged with grass pollen, the relative antianaphylactic activities of salbutamol and DSCG are reversed. Salbutamol is a potent and almost complete inhibitor of the release of histamine and SRS-A. This agrees with results for adrenoceptor agonists in general (Tauber, Kaliner, Stechschulte & Austen, 1972) and with the one published report on salbutamol (Assem & Schild, 1971). The relatively high activity of salbutamol, which acts selectively at β_2-adrenoceptors (Brittain, Jack & Ritchie, 1970) and preliminary studies with adrenoceptor blocking drugs (Skidmore & Vardey unpublished observations) indicate strongly that the β-receptors which modulate the release of histamine and SRS-A from lung are of the β_2-type.

The observation that SRS-A release is more easily inhibited by β-stimulants than is histamine release has been made before (Orange, Kaliner, LaRaia & Austen, 1971). SRS-A, unlike histamine, is not preformed and these authors suggest that increases in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels which mediate the effect of β-stimulants may modulate more than one step in the generation and release of SRS-A.

Unlike salbutamol, DSCG is a weak and partial inhibitor of the release of histamine and SRS-A from human lung. Within individual experiments convincing concentration-dependent responses are rarely observed and only occasionally does inhibition exceed 50%. Although there is some evidence for a decrease in activity with preincubation there is not the rapid and complete loss of inhibition seen when DSCG is incubated with rat mast cells before the addition of antigen. These results agree well with the majority of published findings (Sheard & Blair, 1969; Assem & Mongar, 1970; Orange & Austen, 1972; Broughton, Chaplen, Knowles, Lunt, Pain, Wooldridge, Ford, Marshall, Walker & Maxwell, 1974; Morr, 1978; Sharpe, Ross & Spicer, 1978).

While the activity of salbutamol in human lung and its inactivity in the rat mast cell can be accounted for by the presence or absence of effective β-adrenoceptors on the target cells, the potency of DSCG in the rat and its lack of potency in human lung cannot be easily explained without a clearer understanding of the differences in the biochemistry of the anaphylactic response in the two species. Superficially the responses are the same, requiring the presence of a homocytotropic antibody on the histamine-containing cell, the presence of specific antigen, an energy source, the activation of a labile sequence of biochemical reactions and a requirement for calcium (Kaliner & Austen, 1973; Goth & Johnson, 1975). However, their very different sensitivities to DSCG indicate that they may have different control points or rate-limiting steps. These rate-limiting steps have not been identified. The severity of the antigenic challenge and the extent of histamine release in the human lung *in vitro* are probably far in excess of those experienced in an asthmatic attack and may be responsible for the weak activity of DSCG in this system. However, a similar proportion of total cell histamine is released from rat mast cells during challenge and this release is inhibited efficiently by DSCG.

DSCG is an established prophylactic treatment for allergic asthma and inhibition of the anaphylactic release of spasmogens has been proposed as its mechanism of action (Cox, 1971). However, the evidence that DSCG is a weak inhibitor of anaphylaxis in human lung, the inhibition by DSCG of histamine-induced bronchospasm in asthmatics (Kerr, Govindaraj & Patel, 1970) and the report that DSCG reduces the activity of irritant receptors in the lung of the dog (Jackson and Richards, 1977) suggest that it may have other or additional actions in the asthmatic.

The concentration of salbutamol which inhibits anaphylaxis in human lung is of the same order as

that reported to relax human bronchial muscle (Hedges & Turner, 1969; Svedmyr & Thiringer, 1971) and should easily be achieved *in vivo* especially when administered by inhalation. The present use of salbutamol in allergic asthma is based mainly on its ability to relieve bronchospasm. This property makes the unequivocal evaluation of salbutamol as a prophylactic treatment very difficult. However, the results in this paper and the report of Van As (1975) together with evidence that salbutamol and other β-stimulants may have more than one mechanism of action in exercise-induced asthma (Anderson, Seale, Rozea, Bandler, Theobald & Lindsay, 1976; Ferris, Anderson & Temple, 1978) support the proposal that salbutamol would be expected to be more effective in allergic asthma if given in a prophylactic regimen. Such regular use would help to suppress the inflammatory response in the lungs as well as to achieve the maximum degree of bronchodilation.

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References

ANDERSON, S.D., SEALE, J.P., ROZEA, P., BANDLER, L., THEOBALD, G. & LINDSAY, D.A. (1976). Inhaled and oral salbutamol in exercise-induced asthma. *Am. Rev. Resp. Dis.*, **114**, 493–500.

ASSEM, E.S.K. & MONGAR, J.L. (1970). Inhibition of allergic reactions in man and other species by cromoglycate. *Int. Archs Allergy appl. Immun.*, **38**, 68–77.

ASSEM, E.S.K. & SCHILD, H.O. (1971). Inhibition of the anaphylactic mechanism by sympathomimetic amines. *Int. Archs Allergy appl. Immun.*, **40**, 576–589.

AUSTEN, K.F. (1974). Reaction mechanisms in the release of mediators of immediate hypersensitivity from human lung tissue. *Fedn Proc.*, **33**, 2256–2262.

AUSTEN, K.F. & ORANGE, R.P. (1975). Bronchial asthma: the possible role of the chemical mediators of immediate hypersensitivity in the pathogenesis of subacute chronic disease. *Am. Rev. Resp. Dis.*, **112**, 423–436.

BACH, M.K. & BRASHLER, J.R. (1978). Ionophore A 23187—induced production of slow reacting substance of anaphylaxis (SRS-A) by rat peritoneal cells *in vitro*: evidence for production by mononuclear cells. *J. Immunol.*, **120**, 998–1005.

BRITTAIN, R.T., JACK, D. & RITCHIE, A.C. (1970). Recent β-adrenoceptor stimulants. *Adv. Drug Res.*, **5**, 197–253.

BROUGHTON, B.J., CHAPLEN, P., KNOWLES, P., LUNT, E., PAIN, D.L., WOOLDRIDGE, K.R.H., FORD, R., MARSHALL, S., WALKER, J.L. & MAXWELL, D.R. (1974). New inhibitor of reagin-mediated anaphylaxis. *Nature*, **251**, 651–652.

COLLIER, H.O.J. (1970). Endogenous broncho-active substances and their antagonism. *Adv. Drug Res.*, **5**, 95–107.

COX, J.S.G. (1971). Disodium Cromoglycate. Mode of action and its possible relevance to the clinical use of the drug. *Br. J. Dis. Chest*, **65**, 189–204.

ELLIOT, D.F., HORTON, E.W. & LEWIS, G.P. (1960). Actions of pure bradykinin. *J. Physiol.*, **153**, 473–480.

FERRIS, L., ANDERSON, S.D. & TEMPLE, D.M. (1978). Histamine release in exercise-induced asthma. *Br. med. J.*, **1**, 1697.

FULLARTON, J.R., MARTIN, L.E. & VARDEY, C.J. (1973). Studies on the inhibition of cellular anaphylaxis. *Int. Archs Allergy appl. Immun.*, **45**, 84–86.

GOTH, A. & JOHNSON, A.R. (1975). Current concepts on the secretory function of mast cells. *Life Sci., Oxford*, **16**, 1201–1214.

HEDGES, A. & TURNER, P. (1969). Beta receptors in human isolated smooth muscle. *Br. J. Pharmac.*, **37**, 547–548P.

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

KALEY, G. & WEINER, R. (1971). Prostaglandin E—a potential mediator of the inflammatory response. *Ann. N.Y. Acad. Sci.*, **180**, 338–350.

KALINER, M. & AUSTEN, K.F. (1973). A sequence of biochemical events in the antigen induced release of chemical mediators from sensitized human lung tissue. *J. exp. Med.*, **138**, 1077–1094.

KAY, A.B. & AUSTEN, K.F. (1971). The IgE-mediated release of an eosinophil leucocyte chemotactic factor from human lung. *J. Immun.*, **107**, 899–902.

KERR, J.W., GOVINDARAJ, M. & PATEL, K.R. (1970). Effect of alpha-receptor blocking drugs and disodium cromoglycate on histamine hypersensitivity in bronchial asthma. *Br. med. J.*, **2**, 139–141.

KUSNER, E.J., DUBNICK, B. & HERZIG, D.J. (1973). The inhibition by disodium cromoglycate *in vitro* of anaphylactically induced histamine release from rat peritoneal cells. *J. Pharmac. exp. Ther.*, **184**, 41–46.

LEWIS, R.A., GOETZL, E.J., WASSERMANN, S.I., VALONE, F.H., RUBIN, R.H. & AUSTEN, K.F. (1975). The release of four mediators of immediate hypersensitivity from human leukaemic basophils. *J. Immun.*, **114**, 87–92.

MARTIN, L.E. (1971). Inhibition of cellular anaphylaxis by β-stimulants. *Postgrad. med. J.*, Supplement **47**, 26–30.

MONCADA, S., FERREIRA, S.H. & VANE, J.R. (1973). Prostaglandins, aspirin-like drugs and the oedema of inflammation. *Nature*, **246**, 217–219.

MORR, H. (1978). Effect of disodium cromoglycate (Intal®)

on antigen-induced histamine release from passively sensitized human lung *in vitro*. *Lung*, **155**, 33–38.

NADEL, J.A. (1973). Neurophysiologic aspects of asthma. In *Asthma; Physiology, Immunopharmacology and Treatment*. ed. Austen, K.F. & Lichtenstein, L.M. pp. 29–38. London & New York: Academic Press.

O'NEILL, R. (1971). Function minimization using a simplex procedure. *Applied statistics*, **20**, 338–345.

ORANGE, R.P. & AUSTEN, K.F. (1969). Slow reacting substance of anaphylaxis. *Adv. Immun.*, **10**, 105–144.

ORANGE, R.P. & AUSTEN, K.F. (1972). The immunological release of chemical mediators of immediate type hypersensitivity from human lung. *Prog. Immun.*, **1**, 173–186.

ORANGE, R.P., KALINER, M.A., LARAIA, P.J. & AUSTEN, K.F. (1971). Immunological release of histamine and SRS-A from human lung. II. Influence of cellular levels of cAMP. *Fedn Proc.*, **28**, 1710–1715.

ORANGE, R.P., STECHSCHULTE, D.J. & AUSTEN, K.F. (1969). Cellular mechanisms involved in the release of slow reacting substance of anaphylaxis. *Fedn Proc.*, **28**, 1710–1715.

PIPER, P. & WALKER, J.L. (1973). The release of spasmogenic substances from human chopped lung tissue and its inhibition. *Br. J. Pharmac.*, **47**, 291–304.

SHARPE, T.J., ROSS, J.W. & SPICER, B.A. (1978). The effect of sodium 5,6-dimethyl-2-nitroindanedione on anaphylactic reactions *in vitro*. *Agents & Actions*, **8**, 199–202.

SHEARD, P. & BLAIR, A.M.J.N. (1970). Disodium cromoglycate, activity in three *in vitro* models of the immediate hypersensitivity reaction in lung. *Int. Archs Allergy appl. Immun.*, **38**, 217–224.

SMITH, A.P. & CUTHBERT, M.F. (1972). Prostaglandins and resistance to β-adrenoceptor stimulants. *Br. med. J.*, **2**, 166.

STRANDBERG, K., MATHÉ, A.A. & YEN, S-S. (1977). Release of histamine and formation of prostaglandins in human lung tissue and rat mast cells. *Int. Archs Allergy appl. Immun.*, **53**, 520–529.

SULLIVAN, T.J., PARKER, K.L., EISEN, S.A. & PARKER, C.W. (1975). Modulation of cyclic AMP in purified rat mast cells: II. Studies on the relationship between intracellular cyclic AMP concentrations and histamine release. *J. Immun.*, **114**, 1480–1485.

SVEDMYR, N. & THIRINGER, G. (1971). The effects of salbutamol and isoprenaline on beta-receptors in patients with chronic lung disease. *Postgrad. med. J.*, Supplement **47**, 44–46.

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. Immun.*, **111**, 27–32.

THOMSON, D.S. & EVANS, D.P. (1973). Inhibition of immediate hypersensitivity reactions by disodium cromoglycate. Requirements for activity in two laboratory models. *Clin. exp. Immunol.*, **13**, 537–544.

VAN AS, A. (1975). Beta-adrenergic stimulant bronchodilators. In *New Directions in Asthma*. ed. Stein. pp. 415–431. Park Ridge, Illinois: American College of Chest Physicians.

WILLIAMS, T.J. & MORLEY, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature*, **246**, 215–217.

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