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INHIBITION OF IMMEDIATE HYPERSENSITIVITY REACTIONS IN LABORATORY ANIMALS BY A PHENANTHROLINE SALT (ICI 74,917)

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1 The activity of a new anti-allergic compound, I.C.I. 74,917, has been studied in the rat, mouse and guinea-pig.

2 Following intravenous administration, I.C.I. 74,917 inhibits in a dose-dependent manner passive cutaneous anaphylaxis induced in rats and mice by heat-labile homocytotropic antibody. In rats, its potency is approximately 300 times that of disodium cromoglycate.

3 To achieve maximal inhibition, it is necessary to administer I.C.I. 74,917 at the same time as antigenic challenge; dosing before or after challenge has much less effect.

4 Liberation of histamine, provoked by the antigenic challenge of mast cells passively sensitized *in vitro* by IgE-like antibody, is reduced in the presence of I.C.I. 74,917.

5 Intravenous administration of the compound has no significant effect upon local blueing reactions provoked in the rat by intradermal injection of histamine, 5-hydroxytryptamine or Compound 48/80. It has only a slight effect at high doses upon passive cutaneous anaphylaxis induced in the rat by heat-stable homocytotropic or heterologous (guinea-pig) antibodies.

6 Although not a bronchodilator in the guinea-pig, I.C.I. 74,917 partially inhibits systemic anaphylaxis. A consistent reduction in the severity of antigen-induced bronchospasm was demonstrated in the Konzett-Rössler preparation at doses comparable to those inhibiting passive cutaneous anaphylaxis in the rat. However, there was only slight inhibition of passive cutaneous anaphylaxis in the guinea-pig.

7 I.C.I. 74,917 itself induces bronchospasm when administered to anaesthetized guinea-pigs or to a guinea-pig isolated lung preparation. This effect is reversed by salbutamol, but is not prevented by the prior administration of mepyramine, atropine or methysergide.

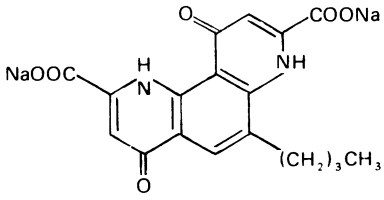
8 These results indicate that in the rat, mouse and guinea-pig, I.C.I. 74,917 is a potent inhibitor of certain types of immediate hypersensitivity reactions.

Introduction

Among the most significant advances in the last decade in the treatment of asthma were the discovery (Cox, 1967) of disodium cromoglycate (DSCG) and its introduction into clinical practice by Howell & Altounyan (1967).

Experimental models demonstrated that DSCG inhibited immediate (Type I) hypersensitivity reactions *in vivo* (Goose & Blair, 1969) and *in vitro* (Sheard & Blair, 1970). These studies suggested that the compound specifically inhibits reactions involving the heat-labile homocytotropic antibody (IgE-like), but more recent work (summarized by Cox, 1971) suggests DSCG is specific for a cell type, the mast cell, rather than for a particular class of immunoglobulin. Even so, the evaluation of DSCG-like compounds *in vivo* depends largely upon their ability to inhibit certain immediate hypersensitivity reactions in suitable laboratory animals. In rats, three models of immediate hypersensitivity reactions may be used. Of these passive cutaneous anaphylaxis (PCA; Ovary, 1964) induced by the IgE-like antibody is a particularly pertinent model as there is now little doubt that in man IgE plays a major role in the pathogenesis of certain types of asthma and hay fever (Ishizaka & Ishizaka, 1970; Johansson, Bennich & Berg, 1972). PCA may also be provoked by a heat-stable homocytotropic antibody, an IgG type (Stechschulte, Austen & Bloch, 1967), but the involvement of human IgG in the pathogenesis of asthma has not yet been unequivocally established (but see Parish, 1973). PCA can also be induced by heterologous antibody, probably reflecting the participation of a Type III (Arthus) reactivity. This type of response may be analogous to that which, in man, is responsible in the lung for allergic reactions of the immune complex type (Pepys, 1973).

Some chemical types other than the chromone-2-carboxylic acids, of which DSCG is an example, also inhibit rat PCA mediated by an IgE-like antibody, e.g. xanthone-2-carboxylic acids (Pfister, Farraresi, Harrison, Rooks, Roszkowski, Van Horn & Fried, 1972) and some 2-nitroindan-1,3-diones (Buckle, Morgan, Ross, Smith & Spicer, 1973). In this paper, we describe the anti-allergic properties in the rat, guinea-pig and mouse of a novel compound, 6-*n*-butyl-2,8-dicarboxy-4,10-dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline (I.C.I. 74,917) as the disodium salt.



I.C.I. 74,917, disodium salt

A preliminary report on this compound has already been published (Evans, Gilman, Thomson & Waring, 1974).

Methods

The guinea-pigs, rats and mice used in this study were from closed colonies (random-bred) of S.P.F. albino strains maintained at Alderley Park for more than 10 years. The animals were housed under standard conditions and allowed free access to food and water.

Administration of drugs

DSCG and I.C.I. 74,917 were dissolved in 0.9% w/v NaCl solution (saline) or buffer as described in the text. Doses and concentrations are expressed in terms of the free acid of both compounds.

Production of antisera

Rat sera containing the heat-labile homocytotropic antibody (IgE-like) against egg albumin (EA; 2 x crystallized, Koch-Light Ltd) were prepared as described by Mota (1964) using *Bordetella pertussis* vaccine (4×10^{10} organisms/ml; Burroughs Wellcome & Co.) as adjuvant. When a 48 h latent period was used, these sera gave in the rat PCA titres between 1 : 8 and 1 : 32. Serum dilutions were chosen so that DSCG at a dose of 2.5 mg/kg intravenously produced a 50% inhibition of PCA; this dilution was usually between 1 : 2 and 1 : 4. Sera containing IgE-like antibody against the nematode parasite *Nippostrongylus brasiliensis* were produced in rats by repeated infection as described by Ogilvie (1967). With a 48 h latent period, a typical serum had a PCA titre of 1 : 640. It was employed at a dilution of 1 : 5 after being standardized against DSCG in the same way as anti-EA serum. The presence of IgE-like antibody was established by the persistence of PCA activity after a latent period in excess of seven days and by lability to heat and mercaptoethanol. Rat sera containing the heat-stable homocytotropic antibody (IgG) were prepared against EA as described by Davies & Evans (1973) using Freund's Complete Adjuvant (Difco). The sera were heated at 56° C for 2.5 h to destroy any IgE-like antibody present. With a 3 h latent period, the sera had a PCA titre of between 1 : 16 to 1 : 32 and were used at a dilution of 1 : 4. Guinea-pig sera were prepared as described by Davies & Johnston (1971) using EA and Freund's Complete Adjuvant.

Passive cutaneous anaphylaxis in the rat

Groups of five rats (150 to 180 g) of either sex were used. The technique was that of Mota (1964) with minor modifications. A latent period of 48 h was used with the IgE-like antibody and of 3 h for the IgG and heterologous (guinea-pig) antibodies. The severity of the PCA reactions was assessed by scoring in arbitrary units, 10 representing maximal severity (an intense blue reaction with a diameter in excess of 2 cm) and zero representing complete inhibition.

Drugs were administered intravenously as solutions in isotonic saline at the same time as antigenic challenge, unless otherwise stated in the text.

Local blueing reactions in the rat

Groups of five rats (150 to 180 g) of either sex received intradermal injections of histamine acid phosphate (BDH Ltd; 100 μg as the base in 0.1 ml of isotonic saline), 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co. Ltd; 10 μg as the base in 0.1 ml of isotonic saline) or Compound 48/80 (Burroughs Wellcome Ltd; 10 and 1 μg in 0.1 ml of isotonic saline). Drugs were administered intravenously, dissolved in saline immediately before the intradermal injections. In one series of experiments either DSCG or I.C.I. 74,917 in isotonic saline (0.05 ml) was administered intradermally immediately following and at the same site as Compound 48/80 (10 and 1 μg in 0.05 ml of isotonic saline). The severity of the reactions was assessed 30 min later as previously described.

Histamine release from rat peritoneal mast cells

Peritoneal cells were harvested from normal rats, washed, and sensitized by incubation with rat serum containing IgE-like antibody (Thomson & Evans, 1973). After thorough washing to remove excess antibody, the cells were challenged with EA in the presence or absence of drug as appropriate and the histamine liberated from the mast cells estimated by an automated assay (Evans, Lewis & Thomson, 1973) based on the fluorimetric procedure of Shore, Burkhalter & Cohn (1959).

The reduction in histamine release in the drug-treated groups is expressed as a percentage of the release in control groups receiving antigen alone. Some 50 to 60% of the net available histamine was released by antigen from these control cells.

Systemic anaphylaxis in the guinea-pig

Groups of female guinea-pigs (250 to 300 g) were sensitized by the intraperitoneal injection of alum-precipitated EA (36 mg). After three weeks, each animal received an intraperitoneal injection of mepyramine maleate (0.1 mg/kg) (May & Baker Ltd), to reduce the severity of anaphylaxis at subsequent challenge. One hour later, antigen (1 mg EA) and drugs were administered intravenously. The animals were then observed and the time of death recorded.

Allergic bronchospasm in the guinea-pig

Guinea-pigs were passively sensitized by intravenous injection of 0.5 mg of a 1 : 50 dilution of guinea-pig anti-EA antiserum. Twenty-four hours later they were anaesthetized with pentobarbitone sodium (70 mg/kg) deeply enough to depress spontaneous respiration. Cannulae were tied into the trachea and jugular vein, and the lungs inflated from a pump at a rate of 68 strokes/min at a constant stroke volume of between 6 and 8 ml. Resting pressure was recorded for 5 min with the apparatus described by Konzett & Rössler (1940) as modified by Davies (1973); following the suggestion of Collier & James (1967), the lungs were over-inflated for 10 s in every 30 seconds. Drug and antigen (1 mg) were then injected into the jugular vein cannula and recording continued for a further 15 minutes.

Passive cutaneous anaphylaxis in the guinea-pig

Groups of five animals including both sexes (300-350 g) were sensitized by an intradermal injection of heat-stable guinea-pig anti-EA antiserum diluted 1 : 1000. Three hours later, the animals were challenged intravenously with antigen (1 mg in 0.5 ml of isotonic saline). Evans blue dye (5% w/v : 0.5 ml) and drug were injected into the ear vein at the same time and the animals killed 30 min later. The severity of the response was assessed by measuring the maximum diameter of the blued areas.

Bronchospasm in the perfused isolated lungs of the guinea-pig

The method was that of Davies (1973). Normal guinea-pigs were killed by a blow on the head and the heart and lungs removed. After removal of the pericardium, a cannula was tied into the pulmonary artery and 10-20 ml of warmed Tyrode solution injected to remove most of the blood, the effluent being allowed to escape through an incision in the auricle. A second cannula was tied in the trachea and the preparation mounted in the apparatus described by Bhattacharya & Delaunois (1955). This apparatus consists essentially of a cylinder made of Perspex closed at the upper end by a lid through which pass tubes connected to the tracheal and arterial cannulae respectively. The lower end of the cylinder is attached to a small bellows pump delivering 7 ml of air per stroke at a rate of 25 strokes/minute. A small flap-valve allows the escape of air. A pressure transducer was attached to a side arm of the tube leading to the tracheal cannula. Compression of air within the cylinder deflates the lungs and withdrawal of air inflates the lungs, the resulting pressure-changes being monitored by the transducer which activates a pen-recorder, thus providing a record of the course of artificial respiration. An aspirator containing Tyrode solution with 2% dextran is attached, via a warming coil, to the arterial cannula, and the vascular system perfused at a rate of 2-3 ml/min, the effluent being allowed to escape through the flap-valve as it opens during the compression stroke of the pump.

I.C.I. 74,917 and other drugs were injected as appropriate into the perfusion fluid close to the arterial cannula and the degree of bronchospasm was estimated from the percentage change in the height of the recording.

Histamine release from guinea-pig lung

The method was a modification of that described by Colquhoun & Brocklehurst (1965). The chopped lung was divided into 300 mg (wet weight) replicates and 4 ml of guinea-pig anti-EA serum, diluted 1 : 60, added. The tissue was sensitized for 2 h at 37° C, washed twice with Tyrode solution and finally resuspended in 4 ml of

the same solution but containing EA (100 μg/ml) and, if appropriate, I.C.I. 74,917. Fifteen min later, the supernatants were removed and 0.15 ml of 9 N perchloric acid added to them. Four ml of 0.4 N perchloric acid was added to the lung fragments and each sample placed in a boiling water bath for approximately 15 minutes. Histamine content was determined as before.

Passive cutaneous anaphylaxis in the mouse

Groups of five mice (20 to 25 g) including both sexes were used (Mota, 1967). Each animal was given an intradermal injection (0.02 ml) of antiserum and challenged 48 h later by an intravenous injection (0.5 ml) containing antigen (2 mg/ml), Evans Blue (0.25% w/v) and, if appropriate, DSCG or I.C.I. 74,917. The severity of the blueing reaction was assessed 30 min later, as previously described.

Results

Passive cutaneous anaphylaxis in the rat

Both I.C.I. 74,917 and DSCG inhibited PCA induced by the IgE-like antibody in a dose-dependent manner (Table 1). The ID_{50}, i.e. the dose required to reduce the PCA response of a group by 50%, was the same in both antigen systems, within the limits of experimental variation; I.C.I. 74,917 was approximately 300 times as potent as DSCG in both systems. To determine whether I.C.I. 74,917 would inhibit PCA if given before or after antigenic challenge, I.C.I. 74,917 (0.05 mg/kg i.v.) was administered to groups of five rats at various times before, and in one case after, antigenic challenge. The results demonstrated the necessity for simultaneous administration of drug and antigen to achieve maximal inhibition (Figure 1).

In contrast to the inhibitory activity in IgE-mediated PCA, neither compound induced a regular dose-dependent inhibition of PCA provoked by the heat-stable IgG antibody or by the heterologous (guinea-pig) antibodies. I.C.I. 74,917 at doses higher than 0.025 mg/kg i.v. and DSCG at doses higher than 5 mg/kg i.v. showed a partial inhibition (20 to 30%) of both responses which was statistically significant ($P \leqslant 0.05$), but increasing the dose did not result in enhanced inhibition.

Dermal blueing reactions in the rat

Cyproheptadine, a potent antagonist of histamine and 5-hydroxytryptamine (5-HT), inhibited in

Table 1 Effect of I.C.I. 74,917 and disodium cromoglycate (DSCG) on rat passive cutaneous anaphylaxis (PCA) provoked by IgE-like antibody.

Compound	Dose (mg/kg i.v.)	No. of animals	Mean score (± s.e. mean)	% inhib. (± s.e. mean)	ID_{50} *(mg/kg) (± 95% C.L.)	Relative potency (±95% confidence limits)
(a) Egg albumin system:						
Control		75	9.6 ± 0.1			
DSCG	1.0	25	8.6 ± 0.2	11 ± 2		
	2.5	25	5.8 ± 0.3	39 ± 3	2.8 ± 0.2	1.0
	5.0	25	3.8 ± 0.3	61 ± 3		
I.C.I.	0.005	25	6.8 ± 0.3	29 ± 3		
74,917	0.01	25	4.6 ± 0.3	52 ± 3	0.009 ± 0.0005	323 ± 30
	0.025	25	3.2 ± 0.2	66 ± 2		
(b) Nippostrongylus brasiliensis *system:*						
Control		25	9.8 ± 0.1			
DSCG	1.0	20	8.1 ± 0.4	18 ± 4		
	2.5	20	5.7 ± 0.5	42 ± 5	3.4 ± 0.2	1.0
	5.0	20	2.9 ± 0.5	71 ± 5		
I.C.I.	0.005	20	7.3 ± 0.4	26 ± 4		
74,917	0.01	20	3.7 ± 0.4	62 ± 4	0.01 ± 0.0006	308 ± 24
	0.025	20	1.5 ± 0.3	85 ± 3		

*The ID_{50} is that dose required to inhibit the PCA response by 50% compared to an untreated control group.

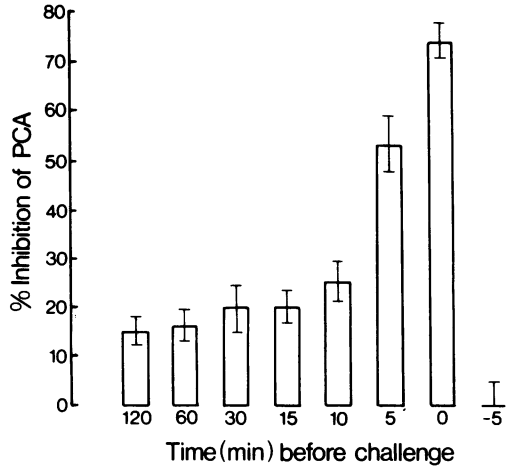


Figure 1 Influence of time of administration of I.C.I. 74,917 on inhibition of passive cutaneous anaphylaxis (PCA). Drug (0.05 mg/kg i.v.) was administered at various times before or, in one case, after antigenic challenge. Results are the mean from five rats, and are expressed in terms of % inhibition compared to an untreated control group. Vertical bars show s.e. mean.

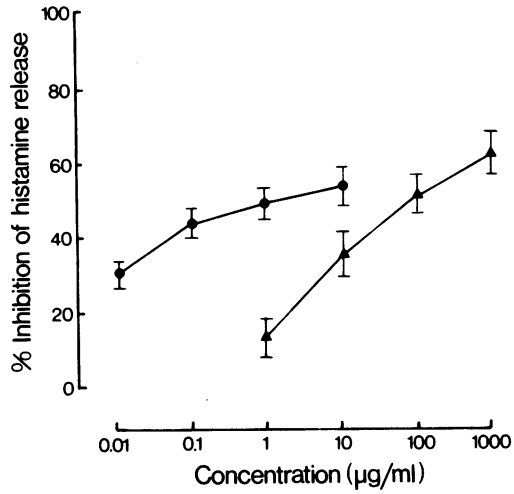


Figure 2 Inhibition of allergic histamine release from passively sensitized rat peritoneal mast cells by I.C.I. 74,917 (●) and disodium cromoglycate (▲). Results are the mean from seven and six experiments, respectively. Vertical bars show s.e. mean.

intravenous doses of 0.25 to 1 mg/kg, in a regular, dose-dependent manner the blueing reactions induced by intradermal injection of histamine (100 μg), 5-HT (10 μg) and Compound 48/80 (0.1 and 10 μg). Neither DSCG nor I.C.I. 74,917 administered intravenously had any significant effect at 10 times the ID_{50} in rat PCA. In contrast, both drugs, administered intradermally, inhibited in a dose-dependent manner blueing reactions provoked by Compound 48/80 (Table 2). Although it was not possible to calculate a meaningful ID_{50} for each compound from these data, I.C.I. 74,917 was approximately 100 times more potent in this model than DSCG.

Histamine release from peritoneal mast cells

Both DSCG and I.C.I. 74,917, when added to sensitized peritoneal mast cells simultaneously with antigen, inhibited allergic histamine release (Figure 2). The dose-response curve for DSCG was steeper and more linear. The effect of I.C.I. 74,917 'levelled off' at approximately 50% inhibition, but for lesser degrees of inhibition this compound, as in the rat PCA test, is clearly much more potent than DSCG.

Allergic reactions and bronchospasm in the guinea-pig

Preliminary experiments established that I.C.I.

Table 2 Inhibition following intradermal administration of disodium cromoglycate (DSCG) and I.C.I. 74,917 of blueing reactions in the rat provoked by Compound 48/80.

Compound	Total dose administered (μg)	No. of animals	% inhib. of blueing response (± s.e. mean) 10 μg*	0.1 μg*
DSCG	50	15	5 ± 5.9	16 ± 7.3
	250	15	21 ± 3.6	33 ± 3.8
	500	15	32 ± 6.7	40 ± 6.2
I.C.I. 74,917	0.5	15	21 ± 4.1	25 ± 4.6
	5	15	38 ± 5.4	41 ± 5.5
	50	15	53 ± 3.7	59 ± 3.1

*Dose of Compound 48/80 injected per site.

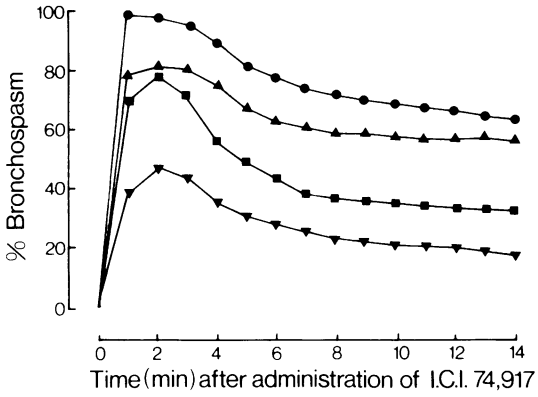


Figure 3 Inhibition of allergic bronchospasm in the anaesthetized guinea-pig. Results are the mean from: untreated controls (●) 20 animals; I.C.I. 74,917-treated, 10 animals/group, (▲) 2.5 μg/kg i.v.; (■) 5 μg/kg i.v. and (▼) 10 μg/kg i.v.

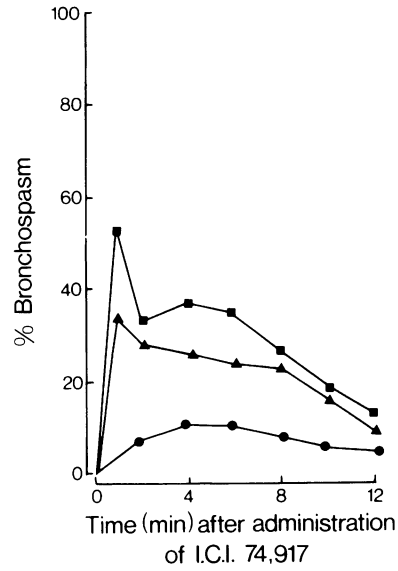


Figure 4 Induction of bronchospasm in anaesthetized guinea-pigs (Konzett-Rössler preparation) by I.C.I. 74,917. Each curve represents the mean measurements from five animals after i.v. administration of 500 μg/kg (■); 50 μg/kg (▲); or 5 μg/kg (●) of compound.

74,917 at doses up to 10 mg/kg intravenously and DSCG at doses up to 25 mg/kg intravenously did not prevent or delay the onset or severity of dyspnoea or collapse provoked by inhalation of an aerosol of histamine or acetylcholine. In contrast, the β-adrenoceptor stimulants isoprenaline and salbutamol showed significant activity in both these models. I.C.I. 74,917 did, however, confer some protection against systemic anaphylaxis (Table 3), but only at doses higher than those inhibiting PCA in the rat; no dose-response relationship was evident. To study this apparent anti-allergic activity more quantitatively, allergic bronchospasm in the anaesthetized guinea-pig (the Konzett-Rössler preparation) was employed (Figure 3). Both the severity of the initial spasm and the duration of the subsequent protracted spasm were reduced in a dose-dependent manner by I.C.I. 74,917. The doses used were comparable to the doses active in rat PCA, but the effect could not be enhanced by employing higher doses (up to

Table 3 Inhibition of allergic reactions in the guinea-pig by I.C.I. 74,917.

(a) *Active systemic anaphylaxis*

Treatment	*Dose mg/kg i.v.*	*No. of animals*	*No. of survivors at 10 min*
Control		28	3
I.C.I. 74,917	0.01	10	7
	0.1	28	16
	1.0	28	14

(b) *PCA (heat-stable antibody)*

Treatment	*Dose (mg/kg i.v.)*	*No. of animals*	*Mean diameter (mm ± s.e. mean)*	*% inhib. (± s.e. mean)*
Control		10	16.2 ± 0.9	
I.C.I. 74,917	0.25	9	11.7 ± 1.2	28 ± 8
	0.5	10	8.5 ± 1.0	48 ± 7
	1.0	10	10.1 ± 0.7	38 ± 6

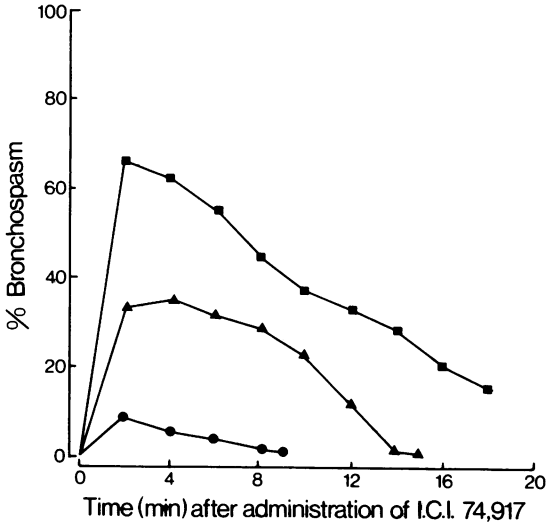


Figure 5 Induction by I.C.I. 74,917 (●) 0.25 μg; (▲) 1 μg; and (■) 5 μg of bronchospasm in the isolated, perfused lung of the guinea-pig. Each curve represents the mean measurements from five preparations.

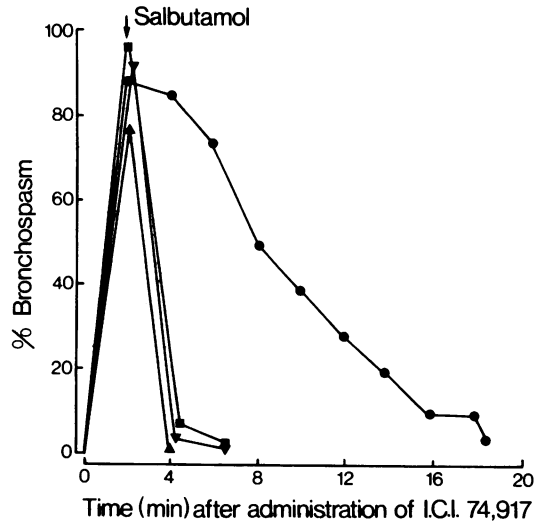


Figure 6 Reversal by salbutamol of bronchospasm induced by I.C.I. 74,917 (10 μg) in the isolated perfused lung of the guinea-pig. Each curve represents the mean measurements from five lung preparations. I.C.I. 74,917 was given alone (●), or followed 2 min later by 0.5 μg (▲); 0.25 μg (■); or 0.1 μg (▼) of salbutamol.

500 μg/kg) of I.C.I. 74,917. Further experiments have shown that to achieve maximal inhibition at a given dose, it is necessary, as in rat PCA, to give drug and antigen simultaneously.

I.C.I. 74,917 itself induced mild reversible bronchospasm both in the anaesthetized guinea-pig (Figure 4) and in isolated perfused lung preparations. As little as 0.25 μg of I.C.I. 74,917/lung produced (Figure 5) a slight but measurable bronchospasm, and 5 to 10 μg produced a maximal response. Increases in dosage of I.C.I. 74,917 did not consistently result in a greater degree of bronchospasm.

When included in the perfusion fluid, mepyramine (1 μg/ml), atropine (1 μg/ml) and methysergide (25 μg/ml) antagonists of histamine, acetylcholine and 5-HT, respectively, did not affect the bronchospasm induced by I.C.I. 74,917. Furthermore, this response was not modified in any way by injection close to the arterial cannula of propranolol (50 μg) or the histamine H_2-receptor blocker, burimamide (1 mg). Bronchospasm initiated by I.C.I. 74,917 was rapidly reversed by injection of salbutamol into the cannula 2 min later (Figure 6).

Guinea-pig PCA was also inhibited by I.C.I. 74,917 (Table 3) but the doses (0.25 to 1 mg/kg i.v.) required were much higher than those inhibiting bronchospasm, and no dose-response relationship was found. In doses up to 50 mg/kg intravenously, DSCG had no activity on these immediate hypersensitivity reactions in the guinea-pig.

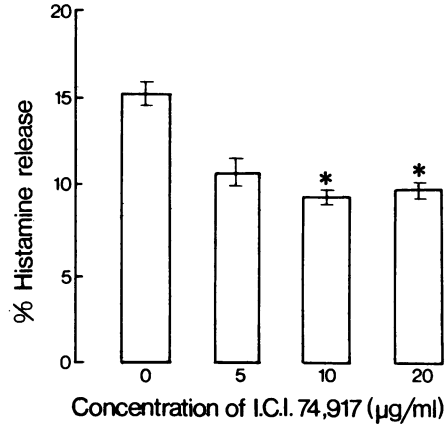


Figure 7 Reduction by I.C.I. 74,917 of histamine release from the chopped, sensitized, guinea-pig lung challenged *in vitro* with antigen. Results are pooled from two experiments performed in triplicate. Vertical bars show s.e. mean. Non-specific release was in all cases less than 1%. * Significant difference from controls ($P < 0.05$).

Preliminary experiments showed that I.C.I. 74,917 in concentrations of 5 to 20 μg/ml inhibited histamine release provoked by antigenic challenge of passively sensitized guinea-pig lung fragments (Figure 7).

Table 4 Inhibition of passive cutaneous anaphylaxis in the mouse induced by an IgE-like antibody.

Compound	Dose (mg/kg i.v.)	No. of animals	Mean score (± s.e. mean)	% inhib. (± s.e. mean)
Control		42	8.3 ± 0.2	
DSCG	10	15	8.1 ± 0.6	3 ± 7.7
	20	15	8.2 ± 0.6	2 ± 7.9
	40	15	8.1 ± 0.3	2 ± 4.8
I.C.I. 74,917	0.5	18	6.1 ± 0.5	27 ± 6
	1.0	20	5.7 ± 0.6	32 ± 7
	2.5	23	2.8 ± 0.4	67 ± 5

Passive cutaneous anaphylaxis in the mouse

Unlike DSCG, I.C.I. 74,917 inhibited PCA in the mouse induced by the IgE-like antibody (Table 4). To achieve this effect, however, it was necessary to employ much higher doses than in the rat and guinea-pig. Inhibition, unlike that in the guinea-pig and rat (IgG-like and heterologous antibodies), was dose-dependent.

Discussion

I.C.I. 74,917 was similar to DSCG in its anti-allergic properties and it was in the rat that this similarity was most obvious. Both compounds inhibited PCA induced by the heat-labile homocytotropic antibody, but I.C.I. 74,917 was approximately 300 times more potent than DSCG in both the EA and *N. brasiliensis* systems (Table 1). To achieve maximal inhibition, it was necessary to administer either compound simultaneously with antigen. When given 10 min before the antigen, inhibition was reduced by some 70%; if given after, no inhibition was found (Figure 1; see also Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard, 1970; Thomson & Evans, 1973). Neither compound inhibited the dermal reactions provoked by local injections of histamine and 5-HT, the two amines known to be responsible for the development of PCA reactions following interaction of IgE-like antibody and antigen (Mota, 1964). It is probable that in the rat, I.C.I. 74,917, like DSCG, prevents the release of inflammatory mediators from sensitized cells. This hypothesis was further supported by the findings that *in vitro,* both compounds when present at antigenic challenge reduced the amount of histamine released from peritoneal mast cells (Figure 2). A high degree of specificity was exhibited by each compound inasmuch as the most noticeable activity was found against responses provoked by IgE-like antibody. Only slight inhibition of reactions to IgG was demonstrated but it was significant, suggesting that specificity was not directed to a particular antibody-type, but more probably to a cell-type, perhaps the mast cell. The bulk of published evidence (summarized by Cox, 1971) would support this suggestion, and in view of the similarity in biological properties of these two compounds in the rat, it is possible that I.C.I. 74,917 acts in a similar manner.

The inhibitory effect on blueing reactions induced by Compound 48/80 was demonstrable only when DSCG or I.C.I. 74,917 was administered intradermally (Table 2). Neither compound was active when given intravenously, which conflicts with the finding of Orr, Hall, Gwilliam & Cox (1971) that DSCG at intravenous doses of 10 and 100 mg/kg inhibited the degranulation of mast cells induced by intradermal injections (0.125, 0.25 and 0.5 μg/site) of Compound 48/80. This discrepancy could possibly be related either to the different end-points employed, i.e. degranulation as opposed to blueing, or the different rat strains. Goose & Blair (1969), however, have reported that DSCG did not inhibit degranulation of mast cells provoked by Compound 48/80.

These studies, therefore, suggest that in the rat the basis of the anti-allergic activity of I.C.I. 74,917 may be the same as that of DSCG, that is, inhibition of mediator release from mast cells.

This similarity between the two compounds does not extend to other species. Thus in the mouse DSCG did not inhibit PCA reactions provoked by an IgE-like antibody (see also Cox *et al.,* 1970), whereas I.C.I. 74,917 did (Table 4). The latter compound may, as in the rat, inhibit the release of amines from murine mast cells, but direct evidence for this is not yet available. In the guinea-pig also, the pharmacological properties of DSCG and I.C.I. 74,917 differed markedly. Although recent reports (Assem, 1973; Taylor & Roitt, 1973) have shown that DSCG inhibits certain allergic reactions in the guinea-pig, no consistent or significant inhibitory activity was found in the present work at intravenous doses up

to 50 mg/kg. In contrast, I.C.I. 74,917 was highly effective in inhibiting allergic bronchospasm provoked by a heat-stable antibody preparation (Figure 3). In preliminary studies the compound partially inhibited histamine release from passively sensitized chopped lung challenged with allergen, but the concentrations required *in vitro* (5 to 20 μg/ml) were high in comparison with those required *in vivo* (2.5 to 10 μg/kg i.v.). Furthermore, I.C.I. 74,917 had little effect upon PCA (Table 3) which could possibly be due to tissue specificity. The bronchospasm produced by I.C.I. 74,917 in the guinea-pig is not of reflex origin as it is demonstrable in an isolated lung preparation. It is unlikely to be related to the release of histamine, 5-HT or acetylcholine from lung tissue, as specific antagonists of these agents did not influence it. The role of other bronchoconstrictor substances, for example the kinins or prostaglandins, cannot be ruled out, but it is possible that the effect may be a direct one upon guinea-pig bronchial muscle. Further studies (unpublished data) have revealed no bronchospasm in the cat, dog, rabbit, rat, marmoset or monkey, or in normal human volunteers, following inhalation of I.C.I. 74,917. It would appear, therefore, that the effect is specific to the guinea-pig. While it is possible that the anti-allergic activity of I.C.I. 74,917 and the bronchospastic activity in guinea-pigs are related, more knowledge of the mode of action of this compound in both situations is required before this concept can be accepted.

In conclusion, therefore, these studies have revealed that I.C.I. 74,917 possesses some properties in common with DSCG, but acts in species and models in which DSCG is inactive. It is possible that I.C.I. 74,917 could represent an entirely different class of anti-allergic agent.

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References

ASSEM, E.S.K. (1973). Inhibition of allergic reactions by cromoglycate and by a new series of compounds (AH6556, AH7079 and AH7725). *Int. Arch. Allergy*, **45**, 708-718.

BHATTACHARYA, B.K. & DELAUNOIS, A.L. (1955). An improved method for the perfusion of isolated lung of guinea-pig. *Arch. int. Pharmacodyn.*, **101**, 495-501.

BUCKLE, D.R., MORGAN, N.J., ROSS, JANET W., SMITH, H. & SPICER, BARBARA A. (1973). Anti-allergic activity of 2-Nitroindan-1,3-diones. *J. Med. Chem.*, **16**, 1334-1339.

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

COLQUHOUN, D. & BROCKLEHURST, W.E. (1965). Passive sensitisation of skin and lung by guinea-pig immunoglobulins. *Immunology*, **9**, 591-611.

COX, J.S.G. (1967). Disodium cromoglycate (FPL670) ("Intal"): a specific inhibitor of reaginic antibody-antigen mechanisms. *Nature, Lond.*, **216**, 1328-1329.

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.

COX, J.S.G., BEACH, J.E., BLAIR, A.M.J.N., CLARKE, A.J., KING, J., LEE, T.B., LOVEDAY, D.E.E., MOSS, G.F., ORR, T.S.C., RITCHIE, JEAN T. & SHEARD, P. (1970). Disodium cromoglycate (Intal). *Adv. drug Res.*, **5**, 115-196.

DAVIES, G.E. (1973). Anti-bronchoconstrictor activity of two new phosphodiesterase inhibitors, a triazolopyrazine (I.C.I. 58,301) and a triazolopyrimidine (I.C.I. 63,197). *J. Pharm. Pharmac.*, **25**, 681-689.

DAVIES, G.E. & EVANS, D.P. (1973). Studies with two new phosphodiesterase inhibitors (I.C.I. 58,301 and I.C.I. 63,197) on anaphylaxis in guinea-pigs, mice and rats. *Int. Arch. Allergy*, **45**, 467-478.

DAVIES, G.E. & JOHNSTON, T.P. (1971). Quantitative studies on anaphylaxis is guinea-pigs passively sensitised with homologous antibody. *Int. Arch. Allergy*, **41**, 648-654.

EVANS, D.P., GILMAN, D.J., THOMSON, D.S. & WARING, W.S. (1974). Inhibition of allergic reactions by a novel phenanthroline, I.C.I. 74,917. *Nature, Lond.*, **250**, 592-593.

EVANS, D.P., LEWIS, J.A. & THOMSON, D.S. (1973). An automated fluorimetric assay for the rapid determination of histamine in biological fluids. *Life Sciences*, **12**, 327-336.

GOOSE, J. & BLAIR, A.M.J.N. (1969). Passive cutaneous anaphylaxis in the rat, induced with two homologous reagin-like antibodies and its specific inhibition with disodium cromoglycate. *Immunology*, **16**, 749-760.

HOWELL, J.B.L. & ALTOUNYAN, R.E.C. (1967). A double-blind trial of disodium cromoglycate in the treatment of allergic bronchial asthma. *Lancet*, **ii**, 539-542.

ISHIZAKA, K. & ISHIZAKA, T. (1970). Biological function of γE antibodies and mechanisms of reaginic hypersensitivity. *Clin. exp. Immunol.*, **6**, 25-42.

JOHANSSON, S.G.O., BENNICH, H.H. & BERG, T. (1972). The clinical significance of IgE. *Prog. clin. Immunol.*, **1**, 157-181.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.*, **195**, 71-74.

MOTA, I. (1964). The mechanisms of anaphylaxis. 1. Production and biological properties of mast cell-sensitising antibody. *Immunology*, **7**, 681-699.

MOTA, I. (1967). Biological characterisation of mouse 'early' antibodies. *Immunology*, **12**, 343-348.

OGILVIE, B.M. (1967). Reagin-like antibodies in rats infected with the nematode parasite *Nippostrongylus brasiliensis*. *Immunology*, **12**, 113-131.

ORR, T.S.C., HALL, D.E., GWILLIAM, J. & COX, J.S.G. (1971). The effect of disodium cromoglycate on the release of histamine and degranulation of rat mast cells induced by Compound 48/80. *Life Sciences*, **10**, 805-812.

OVARY, Z. (1964). Passive cutaneous anaphylaxis. In *Immunological methods*, ed. Ackroyd, J.F. pp. 259-280. Oxford: Blackwell.

PARISH, W.E. (1973). A human heat-stable anaphylactic or anaphylactoid antibody which may participate in pulmonary disorders. In *Asthma. Physiology, immunopharmacology and treatment*, ed. Austen, K.F. & Lichtenstein, L.M. pp. 71-89. New York and London: Academic Press.

PEPYS, J. (1973). Immunopathology of allergic lung disease. *Clinical allergy*, **3**, 1-22.

PFISTER, J.R., FERRARESI, R.W., HARRISON, I.T., ROOKS, W.H., ROSZKOWSKI, A.P., VAN HORN, A. & FRIED, J.H. (1972). Xanthone-2-carboxylic acids, a new series of anti-allergic substances. *J. Med. Chem.*, **15**, 1032-1035.

SHEARD, P. & BLAIR, A.M.J.N. (1970). Disodium cromoglycate. Activity in three *in vitro* models of the immediate hypersentivity reaction in lung. *Int. Arch. Allergy*, **38**, 217-224.

SHORE, P.A., BURKHALTER, A. & COHN, V.H. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmac. exp. Ther.*, **127**, 182-186.

STECHSCHULTE, D.J., AUSTEN, K.F. & BLOCH, K.J. (1967). Antibodies involved in antigen-induced release of Slow Reacting Substance of Anaphylaxis (SRS-A) in the guinea-pig and rat. *J. exp. Med.*, **125**, 127-147.

TAYLOR, W.A. & ROITT, I.M. (1973). Effect of disodium cromoglycate on various types of anaphylactic reaction in the guinea-pig. *Int. Arch. Allergy*, **45**, 795-807.

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.

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