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THE ACTIVITY OF SODIUM CROMOGLYCATE ANALOGUES IN HUMAN LUNG *in vitro*: A COMPARISON WITH RAT PASSIVE CUTANEOUS ANAPHYLAXIS AND CLINICAL EFFICACY

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1 Eleven analogues of sodium cromoglycate have been tested for their ability to suppress histamine release induced by anti-IgE from passively sensitized human lung fragments *in vitro*.

2 With the exception of WY 16922, which released histamine at high concentrations, all inhibited histamine release in a linear dose-related manner.

3 The analogues were 30 to 1500 times more potent than sodium cromoglycate. However, their regression slopes of activity upon log-concentration were only one-third as steep as that for sodium cromoglycate, indicating a possible difference in their mechanism of action.

4 In comparison with sodium cromoglycate, the analogues were more potent in human lung than in rat passive cutaneous anaphylaxis (PCA); there was no quantitative correlation between potencies in the two tests.

5 The human lung model is not predictive of anti-asthmatic activity in man as the six analogues tested clinically are less effective than sodium cromoglycate.

6 These results throw doubt on the use of models of mast cell degranulation in the search for anti-allergic drugs and, possibly, on the relative importance of mast cell degranulation in the pathogenesis of asthma.

Introduction

In the 10 years since its introduction, sodium cromoglycate has been used widely as a prophylactic treatment for all forms of bronchial asthma. However, it has two major disadvantages. Firstly, only 30 to 70% of asthmatics respond to cromoglycate therapy (Brogden, Speight & Avery, 1974) and, secondly, it is not absorbed orally so must be given by inhalation. Therefore, many analogues of sodium cromoglycate have been synthesized with the aim of finding an anti-allergic compound with increased efficacy and activity when given by mouth.

Reagin-mediated passive cutaneous anaphylaxis (PCA) in the rat has been adopted as a primary screen in the search for new anti-allergic drugs following the postulate that the anti-asthmatic activity of sodium cromoglycate is due to its ability to inhibit mast cell degranulation (Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard, 1970) and the finding that rat PCA was readily and reproducibly inhibited by this drug (Goose & Blair, 1969). In this test, many compounds have proved both more active than sodium cromoglycate and effective when given orally (for review see Church, 1978). However, in clinical trials in asthma, no compound has yet been shown to be superior to sodium cromoglycate, the majority being less effective.

With the knowledge that the rat PCA is not predictive of clinical activity, we have tested a series of cromoglycate analogues in IgE-mediated reactions in the target tissue of human asthma, human lung, in the hope that this may provide a more relevant and predictive test of drug efficacy. This paper describes the inhibition of IgE-mediated histamine release from passively sensitized human lung fragments *in vitro* by anti-allergic compounds and compares their activity with that in rat PCA and clinical trials.

Methods

Human lung obtained from lobectomy specimens was chopped finely with scissors, divided into 200 mg replicates and sensitized with 0.2 ml serum, from an allergic donor, in a total of 2 ml Tyrode solution for 18 h at room temperature followed by 1 h at 37°C. Samples were then washed, resuspended in oxygen-

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ated Tyrode solution, brought to 37°C and challenged with anti-IgE (goat anti-human-IgE serum, Miles-Yeda) in a final volume of 0.2 ml. The final dilution of the anti-IgE was 1/1000. After 15 min incubation, the supernatant was removed and the tissue frozen and then thawed to release the remaining histamine. Histamine in the two solutions was assayed spectrofluorimetrically (Evans, Lewis & Thomson, 1973) and the amount released by anti-IgE expressed as a percentage of the original histamine content of the tissue sample. Samples incubated in the absence of antigen were used to correct for spontaneous histamine release. Anti-allergic drugs were added 30 s before challenge with anti-IgE.

Antigen-induced histamine release by lungs from different donors differed markedly, ranging from 6.9 to 34.0% of total lung histamine. In order to compare drug effects over a series of lungs, histamine release induced by anti-IgE in the absence of drug (control) was designated as 100% for each lung. All drug effects were calculated in terms of this figure.

Drugs

Sodium cromoglycate, FPL 52757 (6,8-diethyl-5-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylic acid) and FPL 57787 (6,7,8,9-tetrahydro-5-hydroxy-10-propyl-4*H*-naphtho(2,3-b)pyran-2-carboxylic acid) were kindly donated by Fisons Pharmaceuticals; AH 7725 (7-(2-hydroxyethoxy)-9-oxoxanthene-2-carboxylic acid) by Allen and Hanbury Ltd.; ICI 74917 (bufrolin, 6-*n*-butyl-2,8-dicarboxy-4,10-dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline disodium salt) by ICI Pharmaceuticals Ltd.; doxantrazole (3-(5-tetrazolyl)-thioxanthone-10,10-dioxide) by Burroughs Wellcome; M&B 22948 (2-*o*-propoxyphenyl-8-azapurin-6-one) by May and Baker; PRD-92-EA (5,5-dimethyl-11-oxo-5*H*,11*H*-(2)-benzpyrano(4,3-g)(1)-benzopyran-9-carboxylic acid, ethanolamine salt) by Pharma Research, Canada; RU 31156 (7-(*S*-methyl-sulphonimidoyl)-5-(*n*-hexyl)-xanthen-9-one-2-carboxylic acid, tris-(hydroxymethyl)-aminomethane salt) by Roussel Labs. Ltd.; tixanox (7-(methylsulphanyl)-9-oxanthene-2-carboxylic acid) by Syntex; U 38650 (10-chloro-1,4,6,9-tetrahydro-4,6-dioxopyrido (3,2-g)quinoline-2,8-dicarboxylic acid) by Upjohn; and, WY 16922 (2'-carbamoyl-3'-methoxyoxanilic acid, ethyl ester) by Wyeth. Each compound was dissolved in Tyrode solution immediately before use.

Results

In 15 of 17 experiments, sodium cromoglycate caused a dose-related inhibition of anti-IgE induced histamine release from lung fragments in concentrations up to 200 μM, at which approximately 65% inhibition was observed (Figure 1). Higher concentrations produced little further reduction of histamine release. In the two remaining experiments, cromoglycate failed to cause significant inhibition of histamine release. These were excluded from statistical evaluation.

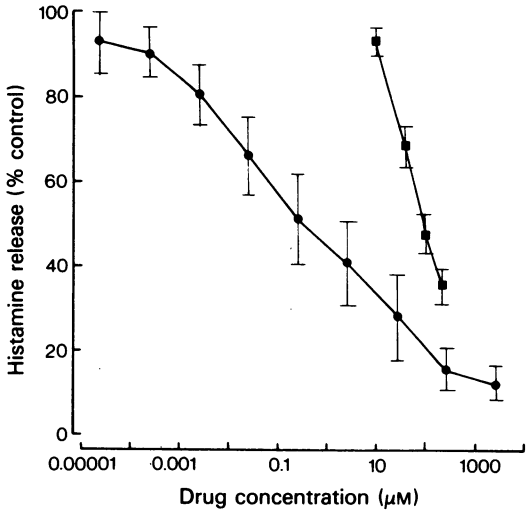

Figure 1 Sodium cromoglycate and ICI 74917 on histamine release from human lung *in vitro*. Each point is the mean of results obtained in 15 experiments for sodium cromoglycate (■) and 6 experiments for ICI 74917 (●); vertical lines show s.e. mean.

The regression line of histamine release upon log concentration of the drug did not differ significantly from linearity ($P > 0.05$). The slope of this line, ± s.e. mean was -45.2 ± 4.2%/log μM. The IC_{50} of sodium cromoglycate (the concentration which reduced control histamine release by 50%), together with standard error limits, was 92.50 (82.24–103.96) μM.

All the cromoglycate analogues tested, with the exception of WY 16922, produced similar effects in this test system (e.g. ICI 74917 in Figure 1). All were significantly more active than sodium cromoglycate: their IC_{50} values were much lower (Table 1) and their highest concentration (250 to 3300 μM) inhibited histamine release to a greater degree (85 to 98%) than sodium cromoglycate. However, the histamine release/−log concentration slopes of the analogues were considerably shallower than that of sodium cromoglycate (Table 2).

WY 16922 differed from the other drugs in that, at concentrations between 0.38 nM and 3.8 μM, it inhibited anti-IgE-induced histamine release, whereas above 3.8 μM, a dose-related increase in histamine release was observed both in the presence and absence of anti-IgE (Figure 2.). The regression slope for inhibition of anti-IgE-induced release was

$-12.6 \pm 1.4\%$/log μM and the IC_{50} of the compound was 0.85 (0.41–1.78) μM.

Discussion

The present experiments show that sodium cromoglycate and structurally related analogues are potent inhibitors of histamine release from passively sensitized human lung fragments *in vitro*. However, the amount of histamine released and the effects of drugs not only vary between lung samples but are also dependent on the source and concentration of the serum used for sensitization and on the nature and strength of the immunological challenge (Coulson, Ford, Marshall, Walker, Wooldridge, Bowden & Coombs, 1977; Church & Gradidge, 1978; Sharpe, Ross & Spicer, 1978). We have attempted to reduce the variability by keeping the sensitizing serum, anti-IgE and experimental conditions constant. Even so, considerable variation remained: in 2 out of 17 experiments sodium cromoglycate was without effect and in the remainder its activity was variable. Similar variability was seen with the analogues. However, weak activity of one compound in a particular experiment did not necessarily mean that others would show weak activity. Similar findings have been reported from clinical trials in which patients who benefited from sodium cromoglycate did not always respond to other anti-allergic drugs and *vice versa* (Pauwels, Lamont & Van der Streaten, 1976).

The finding that the regression slopes of the analogues were only one third as steep as that of sodium cromoglycate (means −13.2 and −45.2%/log μM respectively) suggests that sodium cromoglycate may inhibit histamine release from human lung mast cells by a mechanism that differs in some respects from that of the analogues.

Table 1 IC_{50} values for sodium cromoglycate and analogues against histamine release from human lung induced by anti-IgE

Drug (number of experiments)	IC_{50} (s.e. mean limits) (μM)
Sodium cromoglycate (15)	92.5 (82.3–104.0)
AH 7725 (5)	0.06 (0.04–0.10)
Tixanox (5)	0.22 (0.14–0.37)
PRD-92-EA (5)	0.34 (0.26–0.46)
RU 31156 (5)	0.43 (0.33–0.56)
ICI 74917 (6)	0.45 (0.25–0.83)
FPL 52757 (5)	0.68 (0.52–0.90)
WY 16922 (6)	0.85 (0.41–1.78)
Doxantrazole (6)	1.01 (0.70–1.44)
U 38650 (5)	1.36 (1.02–1.83)
FPL 57787 (5)	2.71 (1.95–3.75)
M&B 22948 (6)	3.02 (2.14–4.25)

The IC_{50} values were calculated from the best fit linear regression lines of inhibition of histamine release against log concentration of drug.

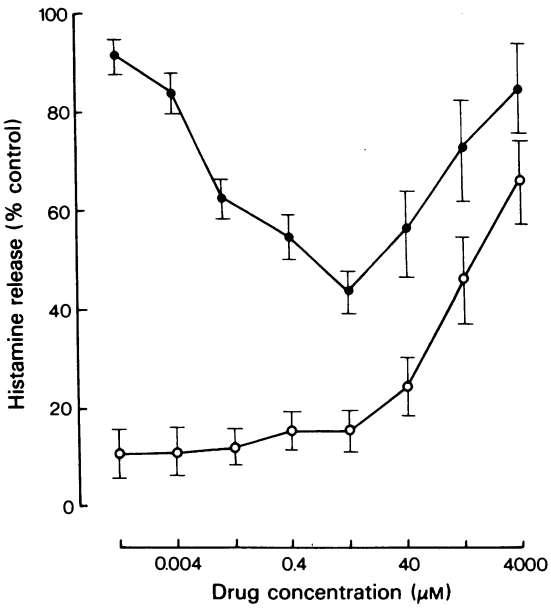

Figure 2 WY 16922 on histamine release from human lung *in vitro*. Each point is the mean of results obtained in 5 experiments: (●) lung fragments challenged with anti-IgE; (○) lung incubated with drug alone; vertical lines indicate s.e. mean.

Comparison with rat passive cutaneous anaphylaxis and clinical efficacy

The cromoglycate analogues were more active in the human lung model than in the rat PCA test. However, their relative potencies in the two tests were not well correlated (Table 3). Although this may suggest a different structure-action requirement for human and rat mast cells, the differences between the two model systems make such conclusions tentative.

The initial hypothesis that inhibition of immunologically induced histamine release from human lung fragments *in vitro* may provide a better predictive model of drug activity in asthma than animal models has proved false. In the clinic, AH 7725, tixanox, PRD-92-EA, ICI 74917 and doxantrazole are at best of comparable efficacy to sodium cromoglycate in bronchial provocation tests, but appear to be inferior in the treatment of asthma (Assem, Evans & McAllen, 1974; Brogden *et al.*, 1974; Poppius & Stenius, 1977; Muittari, Ahonen, Kellomaki, Kuusisto, Lehtinen &

Veneskoski, 1978; Gribben, Harvey & Tattersfield, 1979; Moxham & McAllen, 1979; R. T. Brittain, Allen and Hanbury Research Ltd., personal communication; J. Oran, Syntex Research, personal communication; P. B. Stewart, Boehringer Ingelheim, U.S.A., personal communication). In an open study, ragweed-sensitive patients receiving WY 16922, 400 to 1400 mg orally, required a higher amount of pollen antigen to elicit a weal and flare skin reaction in approximately half of the tests. However, the same doses had no effect on the response to bronchial challenge (M. Deitch, Wyeth Labs., U.S.A., personal communication). Preliminary reports have indicated that FPL 52757 and FPL 57787 have some activity, but results of larger definitive trials have yet to be published (Augstein *et al.*, 1977; Ellul-Micallef & French, 1979). Clinical information is not yet available for M&B 22948 and RU 31156. U 38650 has not been tested clinically.

In the human lung model, all the drugs were considerably more potent than sodium cromoglycate. Furthermore, many anti-histamine-like drugs with

Table 2 Regression slopes for sodium cromoglycate and analogues in human lung *in vitro*

	Slope ± s.e. mean (%/log μM)	*Mean slope* (%/log μM)
Sodium cromoglycate	−45.2 ± 4.2	−45.2
Tixanox	−10.8 ± 1.0	
AH 7725	−11.8 ± 1.5	
Doxantrazole	−12.0 ± 0.9	
ICI 74917	−12.4 ± 1.6	
WY 16922	−12.6 ± 1.4	
FPL 57787	−12.8 ± 1.8	−13.2
M&B 22948	−12.7 ± 1.0	
FPL 52757	−12.7 ± 0.9	
U 38650	−14.9 ± 0.9	
PRD-92-EA	−15.2 ± 1.1	
RU 31156	−17.1 ± 1.0	

Regression slope was calculated as the percentage change in histamine release per incremental log concentration of drug. Results were calculated from 15 experiments with sodium cromoglycate and 5 or 6 experiments with other compounds.

Table 3 Comparative activity of analogues with sodium cromoglycate in the human lung and rat passive cutaneous anaphylaxis (PCA) models

	Potency v. Cromoglycate = 1 in: *Human lung**	*Rat PCA†*	*PCA reference*
Sodium cromoglycate	1	1	Cox *et al.* (1970)
AH 7725	1527	15	Fullarton *et al.* (1973)
Tixanox	414	112	Ferraresi *et al.* (1974)
PRD-92-EA	270	4.5	El-Azab & Stewart (1977)
RU 31156	216	204	Miller & James (1978)
ICI 74917	205	234	Evans & Thomson (1975)
FPL 52757	136	0.12	Augstein *et al.* (1977)
WY 16922	109	1.0	Rosenthale *et al.* (1976)
Doxantrazole	92	0.6	Batchelor *et al.* (1975)
U 38650	68	33	Johnson & Van Hout (1976)
FPL 57787	34	0.11	Augstein *et al.* (1977)
M&B 22948	31	21	Broughton *et al.* (1974)

* Comparative IC_{50} values (μM).

† Intravenous ED_{50} value (mg/kg) for each analogue has been converted to μmol/kg and compared with the ED_{50} value for sodium cromoglycate in the same reference.

negligible anti-asthmatic effects, are also potent inhibitors of histamine release from human lung (Church & Gradidge, 1980). These observations throw great doubt upon the relevance of tests which assess mast cell histamine release in the search for anti-allergic drugs and, possibly, upon the relative importance of mast cell degranulation in the pathogenesis of asthma. They also lead us to ask whether sodium cromoglycate may relieve asthma by effects which many of its analogues or anti-histamines do not share.

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