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RHC 3288 [1-METHYL-2(1,3,4-OXADIAZOL-2(3H)-ONE-5-YL) BENZIMIDAZOLE] AND RELATED COMPOUNDS

NOVEL INHIBITORS OF HISTAMINE RELEASE FROM RAT MAST CELLS AND HUMAN BASOPHILS

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Abstract—RHC 3288 [1-methyl-2(1,3,4-oxadiazol-2(3H)-one-5-yl) benzimidazole] and twenty-five related 5-substituted oxadiazolones have been investigated for their antiallergic activities in three *in vitro* models of anaphylaxis. Sixteen compounds were potent ($I_{50} \leq 50$ μM) inhibitors of antigen-induced release of histamine (AIR) from rat mast cells (RMC), and seven compounds inhibited anti-IgE-induced release of histamine from human basophils ($I_{50} \leq 100$ μM). The antiallergic activity profiles of RHC 3288 and three other compounds in these models have been compared with that of disodium cromoglycate (DSCG). As inhibitors of AIR from RMC, RHC 3288, 3334, 3354 and 3380 were 3 to 10 times more potent than DSCG. In the same model (AIR from RMC), activity profiles of all four RHC compounds were identical to that of DSCG in the following respects: loss of inhibitory activity with increasing preincubation time, tachyphylaxis and cross-tachyphylaxis to each other, and inability to inhibit histamine release stimulated by Ca^{2+} ionophore, dextran + phosphatidyl serine and compound 48/80. RHC 3288, 3334, 3354 and DSCG had no effect in the other two models, histamine release from guinea pig lung mediated predominantly by IgG_1 class of antibodies and anti-IgE-induced histamine release from human basophils. We conclude that RHC 3288 is a potent inhibitor of mediator release with a mechanism of action similar to that of DSCG.

Disodium cromoglycate (DSCG)† is an effective drug in prophylactic treatment of reagin-mediated allergic diseases including hay fever and bronchial asthma [1, 2]. The antiallergic activity of DSCG has been attributed to its ability to inhibit IgE-mediated release of bronchospastic mediators from mast cells, both *in vitro* and *in vivo* [3–5]. However, for the treatment of asthma, DSCG has to be administered directly to the target organ (lung) by insufflation since it is poorly absorbed from the gastrointestinal tract [1]. Therefore, the search has been continued for an orally effective inhibitor of mediator release. Several new orally effective antiallergic agents with a mechanism of action similar to that of DSCG have been discovered and are in various stages of development [6–13].

In this paper we compare the *in vitro* antiallergic activity profile of RHC 3288 (Fig. 1), a new orally active inhibitor of mediator release, and three other related compounds with that of DSCG. In addition, the relationships between the structures of several RHC 3288-related substituted oxadiazolones and their abilities to inhibit *in vitro* immunologic release of histamine from rat mast cells, human basophils, and guinea pig lung slices are also discussed.

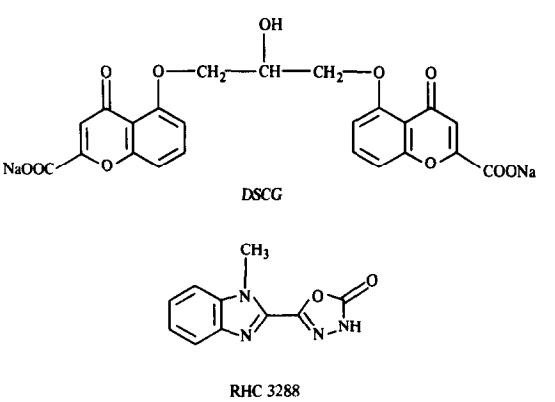

Fig. 1. Structures of RHC 3288 and DSCG.

MATERIALS AND METHODS

(I) *Methods*

(A) *Histamine release from rat mast cells (RMC)*

(1) *Immunologic release of histamine*

(a) *Antigen-induced release of histamine.* The

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† Abbreviations: DSCG, disodium cromoglycate; RMC, rat mast cells; HuB, human basophils; GPL, guinea pig lung slices; and AIR, antigen or anti-IgE-induced release of histamine.

effects of test compounds on antigen-induced release of histamine (AIR) from passively sensitized rat mast cells were determined according to the procedure of Khandwala *et al.* [10]. Briefly, washed RMC were passively sensitized with rat anti-ovalbumin serum *in vitro*, washed, and challenged with ovalbumin to cause release of histamine. Test compounds were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the assay tubes (including controls) was 0.17% which had no effect on either SR or AIR. Test compounds were either added simultaneously with the antigen or preincubated with sensitized RMC for the indicated period of time prior to the addition of antigen. Both spontaneous histamine release (SR) in the absence of antigen and AIR are expressed as percent of total extractable histamine in the cells. The compound activity is expressed as percent inhibition of AIR.

(b) *Tachyphylaxis experiments.* In these experiments inhibition of AIR by RHC 3288 (0-min preincubation) was determined both with untreated sensitized cells (controls) and with sensitized cells incubated first with RHC 3288 for 20 min, followed by the addition of a second dose of RHC 3288, simultaneously with antigen.

(c) *Cross-tachyphylaxis experiments.* Concentration–response curves for RHC 3288 and DSCG (added simultaneously with antigen) were determined both with untreated sensitized cells and with cells preincubated for 20 min with the indicated concentrations of DSCG or RHC 3288 respectively. The first case (preincubation of cells with DSCG for 20 min before the simultaneous addition of RHC 3288 and antigen) is referred to as cross-tachyphylaxis of RHC 3288 to DSCG; the second case (preincubation with RHC 3288 followed by DSCG plus antigen) is referred to as cross-tachyphylaxis of DSCG to RHC 3288. Similarly inhibition of AIR by 10 μM RHC 3334, 3354 and 3380 (added simultaneously with antigen) was determined with untreated sensitized cells and cells preincubated for 20 min with 30 μM DSCG (cross-tachyphylaxis of RHC 3334, 3354 and 3380 to DSCG).

(d) *Additivity of the inhibition of AIR by RHC 3288 and DSCG.* In this experiment, the inhibition of AIR by 0.3, 1 and 3 μM RHC 3288 or DSCG alone and by a combination of matching concentrations of RHC 3288 and DSCG (added simultaneously with antigen) was determined.

(2) *Non-immunologic release of histamine*

(a) *Dextran + phosphatidyl serine-induced release of histamine.* Rat peritoneal cells were washed and used without passive sensitization. The activity of test compound was determined by addition of the compound with 10 μg/ml phosphatidyl serine (PS, 0-min preincubation) or preincubation of the compound with 6 mg/ml dextran and cells for 5 min prior to the addition of PS to initiate histamine release (5-min preincubation) as described by Khandwala *et al.* [11].

(b) *Inhibition of Ca^{2+} ionophore A23187-induced release of histamine.* Rat peritoneal cells were washed and used without passive sensitization. The release of histamine was initiated by the addition of 0.025 μg/ml of Ca^{2+} ionophore A23187. The effect of test compounds on the release of histamine induced by 0.025 μg/ml of ionophore A23187 was determined by adding the compound simultaneously with the ionophore (0-min preincubation), or by preincubation of the compound with cells for 5 min prior to the addition of ionophore (5-min preincubation) as described by Khandwala *et al.* [11].

(c) *Inhibition of compound 48/80-induced release of histamine from rat mast cells.* Rat peritoneal cells were washed and used without passive sensitization. The release of histamine was initiated by the addition of 0.1 μg/ml of compound 48/80. The effect of test compound on compound 48/80-induced release was determined by the addition of the test compound simultaneously with compound 48/80 or by preincubation of the compound with cells for 5 min prior to the addition of compound 48/80 as described by Khandwala *et al.* [11].

(B) *Histamine release from human basophils*

The effect of test compounds on anti-IgE-induced release of histamine from human basophils was determined according to the procedure described by Khandwala *et al.* [10]. Test compounds were dissolved in DMSO. The final concentration of DMSO in assay tubes (including control tubes) was 0.25% which had no effect on SR or AIR. Test compounds were preincubated with basophils (prepared from venous blood from allergic donors) for 5 min prior to the addition of goat anti-human IgE to cause release of histamine.

(C) *Histamine release from guinea pig lung slices*

The effect of test compounds on antigen-induced release of histamine (AIR) from passively sensitized guinea pig lung slices was determined according to the procedure of Khandwala *et al.* [14]. Briefly, guinea pig lung slices were passively sensitized with guinea pig anti-ovalbumin serum *in vitro*, washed, and challenged with ovalbumin to cause release of histamine. Test compounds were dissolved in DMSO. The final concentration of DMSO in the assay tubes (including controls) was 0.25% and had no effect on SR or AIR. Test compounds were preincubated with passively sensitized guinea pig lung slices for 5 min prior to the challenge with antigen as described by Khandwala *et al* [14].

(D) *Data analysis*

Since calculation of percent inhibition of AIR involves errors associated with four variables, i.e. SR and AIR in the presence and the absence of the test compound, a simple calculation of the standard error of the percent inhibition of triplicates is neither appropriate nor adequate to judge the significance of the difference between the values in the presence and absence of the test compound. Therefore, the maximum percentage errors of the measured quantities was calculated by substituting standard deviation of replicate values (nine for control and three with the test compound) into differentials of equations used in calculating results according to Daniels *et al.* [15]. A lack of overlap between maximum errors of two points, therefore, indicates a difference in the means of greater than two standard deviations, i.e. the difference is significant at the 5% level.

(II) *Materials*

All oxadiazolones were synthesized and verified for purity and chemical composition as described

elsewhere.* Disodium cromoglycate was a gift from the Fisons Corp., Bedford, MA. Goat anti-human IgE was obtained from the Meloy Laboratories, Springfield, VA. Dextran, phosphatidyl serine, compound 48/80 and Ca^{2+} ionophore A23187 were purchased from the Sigma Chemical Co., St. Louis, MO. All other chemicals and reagents were purchased commercially and were of reagent trade.

RESULTS

(I) *Comparison of activity profile of RHC* 3288 *with disodium cromoglycate*

(A) *Rat mast cells*

(1) *Inhibition of antigen-induced release of histamine*

(a) *Relative potencies of RHC* 3288 *and DSCG.* Comparative concentration–response curves for the inhibition of AIR by RHC 3288 and DSCG were determined using a common pool of sensitized cells. The results (Fig. 2) show that, when the test compounds were added simultaneously with antigen (0-min preincubation), RHC 3288 (I_{50} = 0.9 μM) was 5–6 times more potent than DSCG (I_{50} = 5.0 μM) as an inhibitor of AIR. The inhibition of AIR by both RHC 3288 and DSCG was not complete. The maximum inhibition of AIR by RHC 3288 (10 μM) was 70% and that by DSCG (30 μM) was 74%. Since RHC 3288 had intrinsic fluorescence at concentrations above 30 μM, its activity at higher concentrations was determined in a separate experiment in which histamine was quantitated by a radioenzymatic method as described by Shaff and Beaven [16]. The I_{50} value of 1.2 μM in this experiment (Fig. 2) for RHC 3288 (0-min preincubation) is in good agreement with the average I_{50} value of 0.9 μM when histamine was quantitated by the automated fluorometric method of Siraganian and Brodsky [17]. The results in Fig. 2 also demonstrate that increasing the concentration of RHC 3288 from 10 to 300 μM did not result in an increase in the inhibition of AIR and that the maximum inhibition of AIR by RHC 3288 did not exceed 76%.

(b) *Effect of time of preincubation on the activity of RHC* 3288 *and DSCG.* The effect of time of preincubation on the inhibition of AIR by RHC 3288 (10 and 3 μM) and DSCG (3 μM) is shown in Fig. 3. The inhibition of AIR by both RHC 3288 and DSCG decreased rapidly with increasing time of preincubation. Both RHC 3288 and DSCG showed maximum inhibition of AIR when they were added simultaneously with antigen. Although in this particular experiment 10 μM RHC 3288 inhibited AIR completely (100%), in eight other experiments the maximum inhibition of AIR by 10 μM RHC 3288 ranged from 62 to 76%. Preincubation of RHC 3288 and DSCG for 10 min with sensitized cells prior to the addition of antigen resulted in 90 and 80% loss of inhibitory activity of RHC 3288 and DSCG respectively. It is clear from these results that the preincubation time profile of RHC 3288 is similar to that of DSCG.

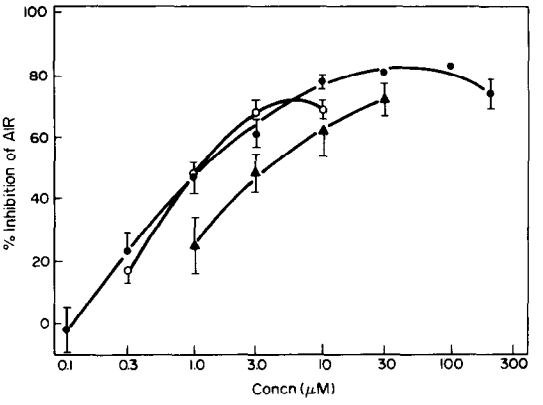


Fig. 2. Composite concentration–response curves for inhibition of AIR by RHC 3288 and DSCG. Compounds were added simultaneously with antigen. Key: (○) RHC 3288, and (▲) DSCG. Each point represents the average ± S.E. of seven experiments carried out in triplicate. The SR and AIR were 3.9 ± 0.6 and 21.6 ± 3.0% respectively (average ± S.E., N = 7). (●) Concentration–response curve for the inhibition of AIR by RHC 3288 using a radioenzymatic method for determining histamine concentrations. Each point represents the average ± S.D. of two experiments carried out in triplicate. The SR and AIR in these experiments were 2.5 ± 0.2 and 30.0 ± 1.5% respectively (average ± S.D., N = 2).

(c) *Tachyphylaxis (self-inhibition).* Since DSCG and other orally active "DSCG-like" agents exhibit tachyphylaxis or self-inhibitory properties [5, 12, 18], the tachyphylactic properties of RHC 3288 were investigated. Fig. 4 shows the results of two such tachyphylaxis experiments carried out with RHC 3288. The results show that, once the sensitized cells were exposed to RHC 3288 for 20 min, subsequent additions of the same concentrations (1, 3, or 10 μM) of RHC 3288 with antigen had no effect on AIR. This loss of sensitivity to the inhibitory properties of the drug is a demonstration of the tachyphylactic properties of RHC 3288.

(d) *Cross-tachyphylaxis of RHC* 3288 *to DSCG.* In this experiment, the effect of pretreatment of sensitized cells with DSCG on the activity of RHC

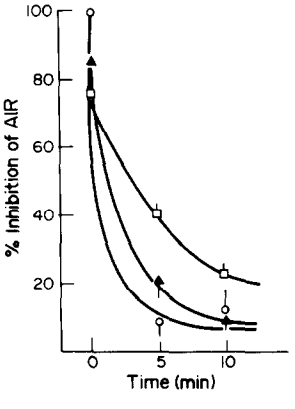


Fig. 3. Effect of preincubation time on the inhibition of AIR. Key: (○) 10 μM RHC 3288, (▲) 3 μM RHC 3288, and (□) 3 μM DSCG. Each point represents the average ± maximum errors of triplicates. The SR and AIR were 5.0 ± 0.2 and 25.1 ± 0.5% respectively.

* J. Musser and F. Huang, U.S. Patent no. 4,268,846, issued May 26, 1981.

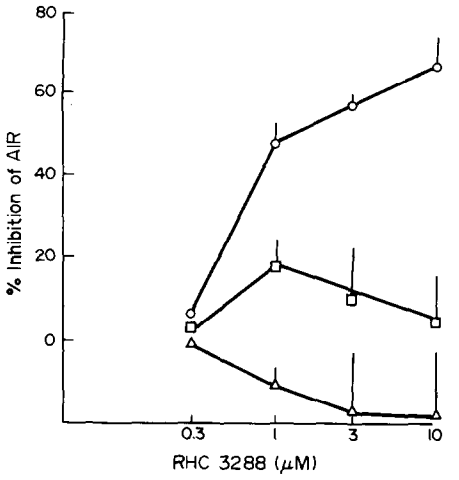


Fig. 4. Tachyphylactic properties of RHC 3288. Key: (○) RHC 3288 added simultaneously with antigen, (□) RHC 3288 preincubated with sensitized RMC for 20 min prior to the addition of antigen, and (△) RHC 3288 preincubated for 20 min with sensitized RMC and a second dose of the same indicated concentration of RHC 3288 added simultaneously with antigen. Each point represents the average ± S.D. of two experiments. The SR and AIR were 4.2 ± 1.1 and 27.4 ± 3.1% respectively (average ± S.D., N = 2).

3288 was determined. The results confirm that DSCG (10 and 30 μM) is tachyphylactic (Fig. 5A) and, once cells were preincubated with 10 or 30 μM DSCG for 20 min, the addition of RHC 3288 simultaneously with antigen had no effect on AIR (Fig. 5B). Furthermore, pretreatment of cells with DSCG (1 μM), which by itself had no effect on AIR, reduced the effectiveness of RHC 3288 by 3-fold as judged from the shift in the I_{50} value of 0.9 μM for the control cells to 2.7 μM for the cells pretreated with 1 μM

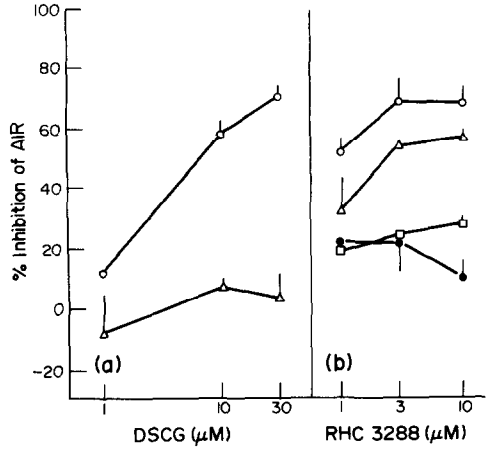


Fig. 5. Cross-tachyphylaxis of RHC 3288 to DSCG. (A) (○) DSCG added simultaneously with antigen, and (△) DSCG preincubated for 20 min with sensitized cells prior to the addition of antigen. (B) RHC 3288 added simultaneously with antigen to untreated cells (○), or cells preincubated with 1 μM DSCG (△), 10 μM DSCG (□), or 30 μM DSCG (●) for 20 min and then challenged with antigen + 1, 3, or 10 μM RHC 3288. Each point represents the average ± maximum errors of triplicates. The SR and AIR were 3.2 ± 0.8 and 23.5 ± 1.6% respectively.

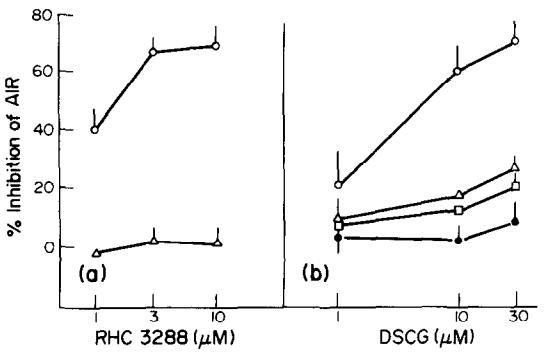


Fig. 6. Cross-tachyphylaxis of DSCG to RHC 3288. (A) (○) RHC 3288 added simultaneously with antigen, and (△) RHC 3288 preincubated with sensitized cells for 20 min prior to the addition of antigen. (B) DSCG added simultaneously with antigen to untreated cells (○), or cells preincubated with 1 μM RHC 3288 (△), 3 μM RHC 3288 (□), or 10 μM RHC 3288 (●) for 20 min and then challenged with antigen + 1, 10 or 30 μM DSCG. Each point represents the average ± maximum errors of triplicates. The SR and AIR were 5.0 ± 0.5 and 27.3 ± 1.1% respectively.

DSCG. RHC 3288 thus exhibited cross-tachyphylactic properties to DSCG.

(e) *Cross-tachyphylaxis of DSCG to RHC* 3288. In this experiment, the effect of pretreatment of sensitized cells with RHC 3288 on the activity of DSCG was determined. The results show that RHC 3288 was tachyphylactic (Fig. 6A), and that, once cells were exposed to 1, 3, or 10 μM RHC 3288, DSCG (10 or 30 μM) added simultaneously with antigen was no longer effective as an inhibitor of histamine release (Fig. 6B). Thus, DSCG is cross-tachyphylactic to RHC 3288.

(f) *Additivity of the inhibition of AIR by RHC* 3288 *and DSCG.* Since neither RHC 3288 nor DSCG completely inhibited AIR, an experiment was car-

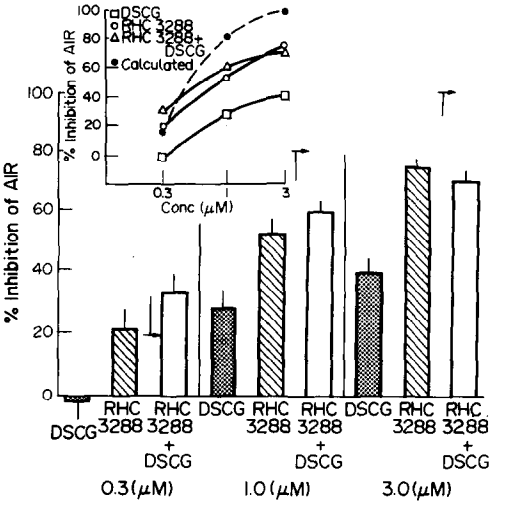


Fig. 7. Lack of additivity of inhibition of AIR by RHC 3288 + DSCG. The compound(s) was added simultaneously with antigen. Each point represents the average ± maximum errors of triplicates. The SR and AIR were 3.0 ± 0.2 and 20.4 ± 0.4% respectively.

ried out to determine if combined doses of RHC 3288 and DSCG would inhibit AIR by 100%. The results (Fig. 7) show that the inhibition of AIR by a combination of 0.3 μM each of RHC 3288 and DSCG (clear bars) was slightly but not significantly more inhibitory than the arithmetical sum (indicated by an arrow) of the inhibition of AIR by 0.3 μM RHC 3288 and DSCG alone. However, the inhibition of AIR by 1 or 3 μM RHC 3288 + DSCG was significantly (no overlap between maximum errors) less than the arithmetical sum of inhibition of AIR by 1 or 3 μM each of RHC 3288 and DSCG. This less than additive effect of RHC 3288 and DSCG is even more evident from concentration–response curves for the inhibition of AIR by 3288, DSCG, and RHC 3288 + DSCG (inset, Fig. 7). The calculated additive concentration–response curve is to the left of the observed curve.

(2) *Non-immunologic release of histamine*

(a) *Dextran + phosphatidyl serine-induced release of histamine.* The dextran + phosphatidyl serine-induced release of histamine in this experiment was 41.4 ± 2.3%. When RHC 3288 was preincubated with cells for 5 min prior to the addition of PS, it did not have any effect. When added simultaneously with PS, RHC 3288 (0.1 to 10 μM) inhibited release of histamine by 20% in a non-concentration-dependent manner. DSCG (1–300 μM) had no inhibitory effect on dextran + phosphatidyl serine-induced release of histamine whether added simultaneously with PS or preincubated with cells prior to the addition of PS (data not shown).

(b) Ca^{2+} *ionophore A23187-induced release of histamine.* Ca^{2+} ionophore A23187-induced release of histamine in this experiment was 78.8 ± 2.5%. RHC 3288 (0.1 to 10 μM) or DSCG (10–100 μM) did not inhibit significantly 0.25 μg/ml ionophore-induced release of histamine with 0- or 5-min preincubation (data not shown).

(c) *Compound 48/80-induced release of histamine.* Compound 48/80-induced release of histamine in this experiment was 58.5 ± 6.5%. Neither RHC 3288 (0.1 to 10 μM) nor DSCG (10–100 μM) significantly inhibited compound 48/80-induced release of histamine with 0- or 5-min preincubation (data not shown).

(B) *Human basophils*

Neither DSCG (1–100 μM, Table 1) nor RHC 3288 (1–300 μM, Table 2) significantly inhibited anti-IgE-induced release of histamine from human basophils.

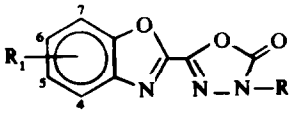
(C) *Guinea pig lung slices*

In this *in vitro* model of anaphylaxis, lung slices were passively sensitized with antibodies consisting predominantly of the IgG_1 class of antibodies [14]. RHC 3288 (0.1 to 10 μM) did not inhibit significantly antigen-induced release of histamine from passively sensitized guinea pig lung slices (Table 2).

(II) *Comparison of activity profiles of RHC* 3334, 3354 *and* 3380 *with disodium cromoglycate*

RHC 3334 (compound 17, Table 2), 3354 (compound 8, Table 1) and 3380 (compound 23, Table 3) were three other oxadiazolones which were potent inhibitors of AIR from RMC. Detailed studies carried out with these three oxadiazolones showed that, (a) when added simultaneously with antigen, RHC 3334, 3354 and 3380 were 9, 10 and 3 times more potent than DSCG as inhibitors of AIR from RMC, respectively, but were ineffective with 5-min preincubation (Tables 1, 2 and 3); (b) they had preincubation time profiles similar to those of RHC 3288 and DSCG, and exhibited tachyphylactic properties (data not shown); (c) all three compounds were cross-tachyphylactic to DSCG (Fig. 8); (d) they had

Table 1. Effects of 2-benzoxazole-oxadiazolones on the release of histamine from rat mast cells, human basophils and guinea pig lung slices*



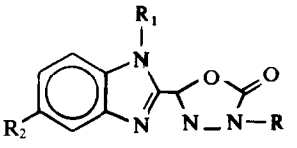
Compound No.	RHC No.	R	R_1	I_{50} (μM) or % inhibition at 100 μM RMC 0 min	RMC 5 min	HuB	GPL
1	3024	H	H	1.2(47)	†(3)	>100	28%
2	3104	CH_3	H	>100	†	>100	
3	3034	$COCH_3$	H	1.4(2)	35	82	13%
4	3079	$COCH{=}C_6H_5$	H	15(2)	8.0(2)	18(3)	20%
5	3299	$CO_2C_2H_5$	H	‡	10†	38	17%
6	3398	$CH_2CO_2C_2H_5$	H	>100	‡	>100	
7	3324	H	4-CH_3	1.0	>100	>100	15%
8	3354	H	5-CH_3	0.4(3)	>100	>300	9%
9	3084	H	5-Cl	0.4		>100	9%
10	3149	H	5-CO_2CH_3, 7-OCH_3	0.3(2)	>100	>300	8%
11	3169	H	5-$CO_2C_2H_5$	1.0		>100	19%
12	3167	H	6-CH_3	5.0(4)			24%
13	3309	$CO_2C_2H_5$	6-CH_3	‡	>100	11	5%
	DSCG			4.5(42)	>300(3)	>100(2)	>500(2)

* Values are the average of the number of experiments (indicated in parentheses); otherwise, values are the average of triplicates.

† Bell-shaped concentration–response curve.

‡ Stimulation of AIR at 100 μM.

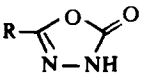
Table 2. Effects of 2-benzimidazole-oxadiazolones on the release of histamine from rat mast cells, human basophils and guinea pig lung slices*

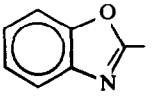
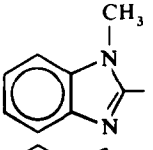
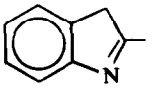
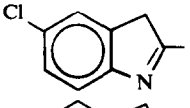
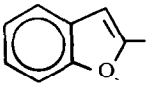
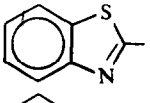


Compound No.	RHC No.	R	R_1	R_2	I_{50} (μM) or % Inhibition at 100 μM RMC 0 min	RMC 5 min	HuB	GPL
14	3288	H	CH_3	H	0.9(9)	†(3)	>300	9%
15	3592	H	3-Morpholino propyl	H	1.0	†	>100	
16	3675	H	4-Chlorophenyl-piperazinyl propanyl	H	†			
17	3334	$COCH_3$	CH_3	H	0.5(3)	>10	>300	4%
18	3308	$CO_2C_2H_5$	CH_3	H	†	>100	†	
19	3483	$CO_2C_2H_5$	CH_3	CH_3	†	†	‡	
20	3489	H	CH_3	CH_3	6.0		‡	

* Values are the average of the number of experiments (indicated in parentheses) carried out in triplicate; otherwise values are the average of triplicates.
† Stimulation of histamine release.
‡ Compound was too fluorescent for analysis of histamine.

Table 3. Effects of 5-substituted oxadiazolones on the release of histamine from rat mast cells, human basophils and guinea pig lung slices*



Compound No.	RHC No.	R	I_{50} (μM) or % Inhibition at 100 μM RMC 0 min	RMC 5 min	HuB	GPL
1	3024		1.2(47)	†(3)	>100	28%
14	3288		0.9(9)	†(3)	>300	9%
21	3392		33(2)	45	72	9%
22	3351		>100	>100	78	2%
23	3380		1.5	†(2)	76	4%
24	3273		22(3)	†(3)		
25	3326		>100	>100	>100	
26	3385	—CH_3	>100	>100	>100	

* Values are the average of the number of experiments (indicated in parentheses) carried out in triplicate; otherwise, values are the average of triplicates.
† Stimulation of histamine release.

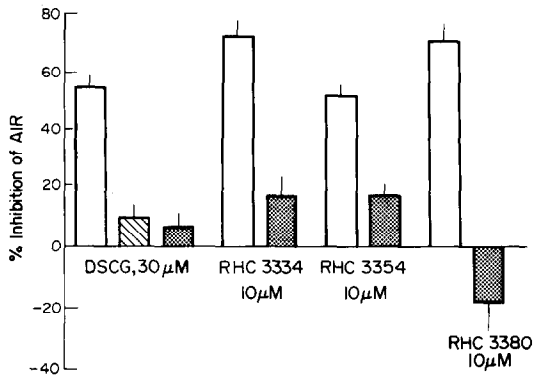


Fig. 8. Cross-tachyphylaxis of RHC 3334, 3354 and 3380 to DSCG. Key: (□) compound added simultaneously with antigen to untreated cells, (▨) DSCG preincubated with sensitized cells for 20 min prior to the addition of antigen, and (▩) compound added simultaneously with antigen to sensitized cells preincubated for 20 min with 30 μM DSCG. Each point represents the average ± maximum error of triplicates. The SR and AIR were 3.1 ± 0.2 and 28.6 ± 2.3% respectively.

no effect on non-immunologic release of histamine from RMC and immunologic release of histamine from guinea pig lung slices; and (e) RHC 3380 (I_{50} = 76 μM) but not RHC 3334 or 3354 inhibited anti-IgE-induced release of histamine from human basophils.

(III) *Activities of oxadiazolones as inhibitors of immunologic histamine release*

The structures and activities of oxadiazolones, substituted in the 5-position with 2-benzoxazoles, 2-benzimidazoles or alkyl, aryl or other heterocyclic substituents, as inhibitors of immunologic histamine release, are shown in Tables 1, 2 and 3 respectively.

(A) *Inhibition of AIR from rat mast cells*

The presence of DMSO had no effect on SR, AIR or the I_{50} value of DSCG as an inhibitor of AIR. In these experiments, SR and AIR were 2.6 ± 0.2 and 23.1 ± 2.6% respectively (average ± S.E., N = 32). We have reported previously [11] that DSCG (3 μM) routinely used as a positive control in each experiment inhibited AIR by 45.2 ± 5.2% (average ± S.E., N = 304) and has an I_{50} value of 4.5 ± 0.5 μM (average ± S.E., N = 96).

(1) 0-*Min preincubation*

Eleven compounds (compounds 1, 3, 7, 8, 9, 10, 11, 14, 15, 17 and 23) were more potent than, DSCG (I_{50} = 5.0 μM) as inhibitors of AIR from RMC. Five other compounds (4, 12, 20, 21 and 24) were potent inhibitors of AIR with I_{50} values of between 5 and 33 μM. The remaining ten compounds were inactive as inhibitors of AIR ($I_{50} \geq 100$ μM) or stimulated AIR significantly.

(2) 5-*Min preincubation*

Generally, when these compounds were preincubated with sensitized RMC for 5 min prior to the addition of antigen, they were not potent inhibitors of AIR. Sixteen of the twenty compounds tested had inhibited AIR by ≤50% or significantly stimulated AIR. The exceptions were N-substituted compounds (compounds 3, 4 and 5, Table 1) and 2-indole oxadiazolone (compound 21, Table 3), which inhibited AIR with I_{50} values of 35, 8, 10 and 45 μM respectively.

(B) *Inhibition of AIR from human basophils*

The presence of DMSO used to solubilize test compounds had no effect on SR, AIR or the I_{50} value of proxicromil as an inhibitor of AIR. In these experiments, SR and AIR were 1.6 ± 0.3 and 31.2 ± 1.5% respectively (average ± S.E., N = 24). We have reported previously [11] that proxicromil used as a positive control in each experiment has an I_{50} value of 54 ± 4 μM (average ± S.E., N = 43).

Most of these compounds (fourteen out of twenty-one tested) were inactive as inhibitors of AIR from HuB (I_{50} > 100 μM or significant stimulation of AIR). The exceptions were four benzoxazole-oxadiazolones in which the N-hydrogen of the oxadiazolone ring was substituted (compounds 3, 4, 5 and 13, I_{50} = 11–82 μM, Table 1) and indole or benzofuran substituted oxidiazolones (compounds 21, 22 and 23, I_{50} = 72–78 μM, Table 3).

(C) *Inhibition of AIR from guinea pig lung slices*

The presence of DMSO used to solubilize test compounds had no effect on SR, AIR or the I_{50} value of isoproterenol as an inhibitor of AIR. In these experiments, SR and AIR were 1.2 ± 0.4 and 27.2 ± 1.3% respectively (average ± S.E., N = 12). We have reported previously [14] that isoproterenol (0.3 μM) routinely used as a positive control in each experiment inhibited AIR by 43.7 ± 4.5% (average ± S.E., N = 24).

At a concentration of 100 μM, none of the seventeen compounds tested inhibited AIR from passively sensitized guinea pig lung slices by ≥28% and thus were not potent inhibitors of histamine release mediated predominantly through the IgG_1 class of antibodies.

DISCUSSION

(I) *Comparison of activity profiles of RHC 3288 with disodium cromoglycate*

The activity profile of RHC 3288, a new orally effective* antiallergic agent, was compared in three most commonly utilized *in vitro* models of anaphylaxis [19] and was found to be similar to that of DSCG in that both compounds inhibited IgE-mediated release of histamine from rat mast cells but not human basophils, and both had no effect on histamine release from guinea pig lung slices, mediated predominantly through the IgG_1 class of antibodies.

The I_{50} value of 5.0 μM reported here for DSCG is in good agreement with average I_{50} values of 4.5 and 4.0 μM reported previously by Khandwala *et al.* [11] and Kusner *et al.* [5] respectively. When added simultaneously with antigen, RHC 3288 was approximately 5–6 times more potent than DSCG as an inhibitor of AIR. Like DSCG, RHC 3288 did not completely inhibit AIR and was effective only when added simultaneously with antigen. Both RHC 3288 and DSCG lost inhibitory activity rapidly with increasing preincubation time prior to the addition of antigen. RHC 3288, like DSCG, exhibited tachyphylaxis and both drugs were cross-tachyphy-

* Personal communication, Dr. P. Sonnino-Goldman; Revlon Health Care Group R & D.

lactic to each other, suggesting that both RHC 3288 and DSCG inhibit AIR from RMC by a similar, if not identical, mechanism. This conclusion is further supported by the results of the additivity experiment in which the response of both drugs added together was less than calculated additive responses of each drug added individually, and less than complete, i.e. <100%. Thus, if RHC 3288 and DSCG were inhibiting AIR by a different mechanism, then the inhibition of AIR by a combined dose of 3 μM RHC 3288 and 3 μM DSCG should have been 100%. However, the observed inhibition was only 68%, which was not different from 70% inhibition of AIR by 3 μM RHC 3288 by itself.

The molecular basis for the inhibition of histamine release from mast cells by DSCG and DSCG-like antiallergic agents has been proposed recently. DSCG and similar antiallergic drugs interact with a surface receptor [20] and elicit the phosphorylation of a mast cell protein having an apparent molecular weight of 78,000 daltons [21]. The same protein is phosphorylated during secretion in the absence of DSCG as well, but only at the end of the secretory process, perhaps as part of the cellular mechanism for turning off exocytosis [22]. Indeed, the 78K protein may be part of the cellular calcium gate. Theoharides *et al.* [22] also showed that the radioactive phosphate incorporated into the 78K protein and the inhibitory effect on histamine release disappeared at similar rates as a function of time of preincubation of the cells with DSCG. A second exposure to DSCG of cells that were pretreated with DSCG failed to promote phosphorylation of the protein, thus mirroring the tachyphylactic properties of the drug at the molecular level. Our interpretation of data presented here for RHC 3288, which demonstrate the loss of activity with increasing preincubation time, the tachyphylactic properties and the cross-tachyphylaxis of RHC 3288 and DSCG to each other, is that RHC 3288 and DSCG interact at the same receptor on the mast cell and that, by analogy with DSCG, RHC 3288 probably elicits the phosphorylation of 78K mast cell protein as well, although we can offer no direct proof of this.

A further comparison of the activity profiles of RHC 3288 and DSCG in the rat mast cells model revealed that both RHC 3288 and DSCG were potent inhibitors of only the immunologic release of histamine from RMC. When histamine release from RMC was induced by the non-immunologic secretogogues dextran + phosphatidyl serine, compound 48/80 or Ca^{2+} ionophore A23187, neither RHC 3288 nor DSCG had any significant effect. The inability of DSCG to inhibit release of histamine from RMC by the three non-immunologic secretagogues is in contrast to previously reported results [18, 23–25]. We have reported previously the inability of DSCG to inhibit non-immunologic release of histamine and have discussed possible reasons for these conflicting results [11]. The inability of DSCG to inhibit the compound 40/80-induced release of histamine is particularly surprising, since compound 48/80 was the secretogogue used by Theoharides *et al.* [22] to demonstrate that DSCG may inhibit histamine release by phosphorylating 78K protein essential for the secretory process. The reasons for discrepant results with DSCG as an inhibitor of compound 48/80-induced release of histamine [11, 18, 23, 26–29] are not clear.

Similarly, the antiallergic activity profiles of RHC 3334, 3354 and 3380 are identical to that of DSCG with respect to preincubation time profile, tachyphylaxis, cross-tachyphylaxis to DSCG, and the lack of effect on non-immunologic release of histamine in the RMC model, suggesting that RHC 3334, 3354 and 3380 also inhibit AIR from RMC by a mechanism similar to that for DSCG.

(II) *Structure–activity relationships*

The 5-substituted-oxadiazolones described in this paper show some interesting relationships between the chemical substitution at the 5-position of the oxadiazolone moiety and the ability to inhibit immunologic release of histamine from rat mast cells (0-min preincubation) and human basophils. A heterocyclic substituent is essential for the inhibition of AIR from RMC or HuB; a simple alkyl or aryl substituent is inactive (compare compounds 25 and 26 vs 1, 14, 21, 22 or 24, Table 3). In oxadiazolones substituted in the 5-position by either a 2-benzoxazole (Table 1) or a 2-benzimadazole (Table 2), substitution of the oxadiazolone N-hydrogen by an alkyl or ethylacetate group resulted in the loss of inhibitory activity.

Substitution of one or more of the hydrogens of the phenyl ring by functional groups generally resulted in compounds either equal to or more potent than the parent compound without affecting its activity profile as an inhibitor of histamine release from rat mast cells (compare activity profiles of RHC 3354 with that of RHC 3288). In the 2-benzoxazole but not in the 2-benzimidazole series, the substitution of the oxadiazolone-N-hydrogen by an acetyl group resulted in a compound which inhibited AIR from RMC with 5-min preincubation. From the data presented in Tables 1–3 and the activity profile of RHC 3380, the following observations can be made about the relationship between the ability of a compound to inhibit AIR from HuB and its effect on AIR from RMC: (a) compounds which did not inhibit AIR from RMC, when added simultaneously with antigen ($I_{50} > 100$ μM), also had no effect on AIR from HuB, (b) inhibition of AIR from HuB was a necessary but not sufficient condition for a compound to inhibit AIR from RMC with 5-min preincubation (e.g. compounds 3, 4, 5, and 21; compounds 22 and 23), and (c) the ability of a compound to inhibit AIR from HuB did not necessarily confer activity as an inhibitor of histamine release from RMC; for example, RHC 3380 (compound 23) inhibited AIR from HuB with an I_{50} value of 76 μM but its activity profile as an inhibitor of histamine release from RMC was identical with that of RHC 3288, 3334, 3354 and DSCG, none of which had any effect on AIR from HuB. This last observation is not unique, as Augstein *et al.* [13] have reported that proxicromil (a chromone derivative structurally similar to DSCG) exhibits tachyphylaxis and is cross-tachyphylactic to DSCG in the RMC model but, unlike DSCG, proxicromil is capable of inhibiting AIR from HuB with an I_{50} value of 60 μM. We have reported previously an I_{50} value of 54 μM for proxicromil as an inhibitor of AIR from HuB [11].

We conclude that oxadiazolones, in general, are potent inhibitors of IgE-mediated release of histamine from rat mast cells with a mechanism of action similar to that of DSCG. One of the orally active compounds, RHC 3288, may be useful in the prophylactic treatment of allergic diseases including bronchial asthma.

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