The role of hydrogen peroxide in basophil histamine release and the effect of selected flavonoids

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Studies on hydrogen peroxide (H_2O_2)–induced histamine release from human basophils indicate that H_2O_2 is a weak stimulus of histamine release, that the release process is Ca^{2+} and energy-dependent, and that histamine release is not influenced by theophylline (in keeping with previous observations with rat mast cells). Low concentrations of H_2O_2 appeared to augment and high concentrations to inhibit histamine release induced by anti-IgE. However, the inhibitory effect of high concentrations of H_2O_2 were completely abrogated by catalase, which destroys H_2O_2, and thus indicates that basophils retain immunologic responsivity and are not irreversibly effected by high concentrations of H_2O_2. Leukocyte suspensions relatively enriched in monocytes, lymphocytes, basophils, neutrophils, and neutrophils plus eosinophils were prepared by Percoll-gradient centrifugation. Anti-IgE stimulated H_2O_2 formation only in the fraction richest in basophils. Opsonized zymosan, on the other hand, stimulated H_2O_2 generation in both the basophil and monocyte fractions, indicating activation of both monocytes and basophils by this stimulus. Mixtures of basophil-containing leukocyte suspensions plus purified neutrophils and opsonized zymosan stimulated histamine release in proportion to concomitant generation of H_2O_2. Addition of catalase reduced histamine release under these conditions, whereas scavengers of other toxic oxygen derivatives (superoxide dismutase, α-tocopherol, D-mannitol) had little or no effect on histamine release. These findings suggest that neutrophil-derived H_2O_2 can cause basophil histamine release in mixed populations of activated leukocytes. Three naturally occurring flavonoids, quercetin, apigenin, and taxifolin (dihydroquercetin) were examined for their effect on anti-IgE-induced histamine release and H_2O_2 generation in basophil-containing leukocyte suspensions. Quercetin and apigenin (5 to 50 μM) inhibited histamine release in a concentration-dependent manner. However, all three flavonoids inhibited the generation of H_2O_2. Since taxifolin inhibited H_2O_2 generation but had little inhibitory effect on histamine release indicates that H_2O_2 generation is not a prerequisite for anti-IgE-induced histamine release. These findings also suggest that certain flavonoids may be useful biochemical probes into the mechanisms of basophil histamine release. (J ALLERGY CLIN IMMUNOL 78:321-8, 1986.)

During phagocytosis by polymorphonuclear leukocytes and monocytes, changes in oxidative metabolism are known to occur. These changes include increased oxygen consumption, generation of O_2^-,[1-3] H_2O_2,[4, 5] $OH^{\cdot}$,[6] $^1O_2$,[7, 8] hypochlorous acid,[9, 10] and increased glucose catabolism through the hexose monophosphate pathway.[11-13] The metabolism of exogenous glucose to CO_2 in rat mast cells was demonstrated to be stimulated during histamine release induced by immunologic or nonimmunologic stimuli, and the increased CO_2 production continued after the release of histamine was complete.[14] Rat and human mast cells as well as human leukemic basophils exhibited parallel release of O_2^- and histamine induced by anti-IgE.[15] Furthermore, rat peritoneal mast cells release histamine on exposure to xanthine oxidase plus hypoxanthine and also after exposure to H_2O_2.[16, 17]

It is now well recognized that certain plant flavo-

Abbreviations used
H_2O_2: Hydrogen peroxide
O_2^-: Superoxide anion
$OH^{\cdot}$: Hydroxyl radical
$^1O_2$: Singlet oxygen
SOD: Superoxide dismutase

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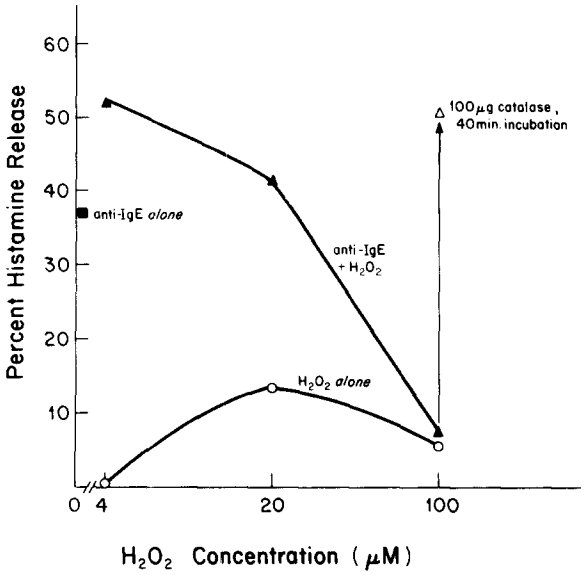

FIG. 1. Histamine-releasing effect of H_2O_2 and the effect of H_2O_2 on anti-IgE-induced histamine release. Basophil-containing leukocyte suspensions were incubated with H_2O_2 alone or with H_2O_2 and anti-IgE (20 μg) for 40 minutes at 37° C. Catalase (100 μg) was added to cells that had been incubated with anti-IgE and 100 μM of H_2O_2 for 40 minutes, and incubation was continued for an additional 40 minutes that led to a normal histamine-release response to anti-IgE *(arrow)*. Results displayed are representative of one of three such experiments.

noids inhibit the stimulated release of histamine from rat mast cells[18-20] and human basophils.[21-23] Certain flavonoids also inhibit the generation of chemiluminescence and O_2^- in purified stimulated human neutrophils.[24]

In this study we investigated the effect of H_2O_2 on histamine release from basophils and determined some biochemical properties of H_2O_2-induced histamine release. In addition, we studied opsonized zymosan-induced histamine release from human basophils mixed with autologous cell fractions enriched in neutrophils or eosinophils and assessed the contribution of H_2O_2 and O_2^- to this process. Finally, the effect of three structurally unique flavonoids on these processes was examined.

MATERIAL AND METHODS

Chemicals

Percoll and Dextran 250 were obtained from Pharmacia Fine Chemicals, Piscataway, N. J. Scopoletin, horseradish peroxidase, ferricytochrome C (type VI), zymosan, SOD, catalase, D-mannitol, H_2O_2 (30%), theophylline, and 2-deoxy-D-glucose-6-phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. *Dermatophagoides farinae* allergen extract was obtained from Torii Co., Japan. Anti-IgE was a gift from Dr. John Wypych, Allergy Research Laboratory, Buffalo General Hospital. The flavonoids were obtained from the following sources: quercetin, Aldrich Chemical Co., Milwaukee, Wis.; apigenin and taxifolin, Fluka, Hauppauge, N. Y. The flavonoids were dissolved in dimethyl sulfoxide to prepare 10 mmol/L stock solutions and diluted in buffer to achieve final concentrations of 5 to 50 μM. The final concentration of dimethyl sulfoxide in cell suspensions did not exceed 0.5%, a concentration that does not affect basophil histamine release.

Cell separation

Each type of leukocyte was isolated by modification of the Percoll technique.[25] Heparinized venous blood from patients allergic to *D. farinae* was mixed with 6% dextran (Dextran 250) in 0.15 mol/L of NaCl and left at room temperature for 40 minutes. The dextran-plasma-leukocyte suspension was collected, and the cells were washed once with saline and resuspended in Percoll solution (density 1.064 gm/ml) with 5% fetal bovine serum at cell concentration of about 30×10^6/ml. A discontinuous Percoll gradient was prepared in polypropylene tubes by adding Percoll solutions of varying density (grams per milliliter) sequentially to the tubes as follows: 3 ml of 1.099, 3 ml of 1.091, 3 ml of 1.078, and 3 ml of 1.071, and finally on top, 1 ml of the cell suspension was carefully overlaid. The tube was centrifuged at 1600 × g for 20 minutes at room temperature. The cells were collected from each layer, contaminating erythrocytes were lysed by hypotonic shock, and cell morphology and type was determined by staining with Giemsa, Alcian blue, and α-naphthyl acetate. The cells were suspended in phosphate-buffered saline, pH 7.4, containing (per liter) 8 gm of NaCl, 0.2 gm of KCl, 1.15 gm of Na_2HPO_4, 0.2 gm of KH_2PO_4, 0.13 gm of $CaCl_2$, 0.1 gm of $MgCl_2$, 0.8 gm of glucose, and 3 gm of human serum albumin. Cell viability for each fraction was more than 95% as determined by trypan blue dye exclusion. The differential cell count for each fraction is provided in the appropriate section of Results.

Measurement of H_2O_2 generation

The formation and release of H_2O_2 into the extracellular medium at 37° C was measured every 5 minutes with the horseradish peroxidase-mediated extinction of scopoletin fluorescence during its oxidation.[4] The scopoletin concentration was 4 μM, and that of HPO was 22 μM. The results were calculated as the maximal rate of H_2O_2 release (nanomoles per minute).

Measurement of O_2^- generation

The generation and release of O_2^- into the medium at 37° C was determined every 5 minutes by measurement of reduction of 50 μM ferricytochrome C.[4] Results are expressed as maximal rate of O_2^- generation (nanomoles per minute).

Measurement of histamine

Histamine was determined by the spectrophotofluorometric method of May et al.[26] The results are expressed as

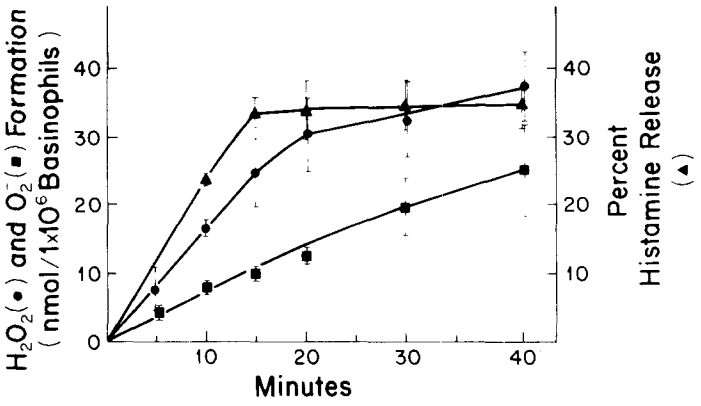

FIG. 2. Time course of histamine release and generation of H_2O_2 and O_2^- from basophil-containing leukocyte suspensions exposed to anti-IgE (20 μg) for 40 minutes at 37° C.

TABLE I. H_2O_2 generation from leukocyte fractions under different conditions

	Cell fraction				
	Monocyte	**Lymphocyte**	**Basophil**	**Neutrophil**	**Neutrophil plus eosinophil**
No. of experiments	3	3	6	6	4
Differential cell count (%)					
Monocyte	15.0 ± 2.5	1.2 ± 0.6	1.3 ± 0.5	0	0
Lymphocyte	85.0 ± 2.5	98.7 ± 0.6	96.5 ± 0.4	0.7 ± 0.8	8.9 ± 13.9
Basophil	0.03 ± 0.04	0.03 ± 0.04	2.0 ± 0.3	0	0
Neutrophil	0	0	0.1 ± 0.2	97.9 ± 1.2	43.3 ± 9.1
Eosinophil	0	0	0	1.4 ± 1.2	47.8 ± 10.9
	H_2O_2 generation picomoles per 10^6 cells per minute				
Additions					
None	0	0	0	43.6 ± 41.1	59.4 ± 43.7
Anti-IgE	0.1 ± 0.1	0.1 ± 0.2	18.6 ± 5.4	45.8 ± 32.7	66.0 ± 12.9
Opsonized zymosan	15.0 ± 6.7	0.2 ± 0.3	13.3 ± 0.4	163.5 ± 99.3	150.3 ± 93.5

percent histamine release relative to the total cellular histamine content as follows:

$$\text{Histamine release (\%)} = \frac{E - B}{C - B} \times 100$$

where E is the fluorometric reading of the experimental sample, B is the reading of samples with cells and buffer, and C is the reading of the "complete" cell mixture (cells disrupted with $HClO_4$). H_2O_2 did not interfere with the analytical technique for histamine.

Statistical analysis

Data are expressed as means ± SD. Significance was determined by the paired Student's t test.

Preparation of opsonized zymosan

Opsonized zymosan was prepared as previously described.[24]

RESULTS

Characteristics of H_2O_2-induced basophil histamine release and the interaction of H_2O_2 with anti-IgE-induced histamine release

H_2O_2 proved to be a relatively weak stimulus to histamine secretion. Fig. 1 illustrates the effect of 4, 20, and 100 μM of H_2O_2. Maximum histamine release was observed at 20 μM of H_2O_2, and a decrease in histamine release was noted with 100 μM of H_2O_2. However, in studies of the interaction of H_2O_2 with an immunologic stimulus to histamine release, i.e., anti-IgE, it was found that 4 μM of H_2O_2 enhanced anti-IgE-induced histamine release, whereas 20 μM of H_2O_2 had no significant effect, and 100 μM of H_2O_2 actually decreased anti-IgE-induced histamine release from 37.2% to 7.7% as illustrated in Fig. 1. A similar histamine release–augmenting effect of 4

TABLE II. Effect of opsonized zymosan and mixtures of opsonized zymosan with neutrophils or eosinophils on basophil histamine release and the inhibitory activity of substances acting on toxic oxygen derivatives

Additions to basophil-containing cell fractions*						
Opsonized zymosan	**Neutrophils**	**Eosinophils**	**SOD**	**Catalase**	**α-tocopherol**	**D-mannitol**
+						
	+					
+	+					
+	+		+			
+	+			+		
+	+		+	+		
+	+				+	
+	+					+
+	+		+		+	+
+	+		+	+	+	+
		+				
+		+				

nd = Not done.

*1 × 10^6 cells of the basophil fraction (basophils, 3.1 ± 1.1; monocytes, 3.0 ± 0.5; lymphocytes, 93.8 ± 1.6; and neutrophils, 0.1 ± 0.05%) with or without 2.5 × 10^6 cells of a neutrophil-enriched suspension (neutrophils, 98.8 ± 0.3; lymphocytes, 0.9 ± 0.6; and eosinophils, 0.3 ± 0.3%) or eosinophil-enriched suspension (eosinophils, 89.0 ± 7.6; neutrophils, 9.8 ± 6.4; and lymphocytes, 1.2 ± 1.2%) were suspended in a final volume of 1.0 ml, incubated at 37° C for 40 minutes with opsonized zymosan (1 mg/ml final concentration), and centrifuged, and supernatant histamine was determined. Final concentration of each scavenger was SOD, 50 μg/ml; catalase, 100 μg/ml; α-tocopherol acetate, 450 μg/ml; and D-mannitol, 1 mmol/L. Data represent mean (± SD) of two experiments with duplicate or triplicate determinations.

†$p < 0.05$ comparing opsonized zymosan with opsonized zymosan plus neutrophils.

‡$p < 0.025$ comparing opsonized zymosan with opsonized zymosan plus neutrophils plus SOD.

§$p < 0.05$ comparing opsonized zymosan plus neutrophils with opsonized zymosan plus neutrophils plus all four scavengers.

TABLE III. Effect of catalase on the histamine-releasing activity present in the supernatant of neutrophils exposed to opsonized zymosan

Stimulus	Histamine release (%)
Supernatant (untreated)	15.1
Supernatant plus catalase	0.6

μM of H_2O_2 and inhibitory effect of 100 μM of H_2O_2 was also observed with *D. farinae*–induced histamine release (data not presented).

To determine whether the inhibitory effect of 100 μM of H_2O_2 on anti-IgE-induced histamine release was reversible, catalase (100 μg/ml) was added at the end of the initial 40-minute incubation and the incubation was continued for an additional 40 minutes. As illustrated in Fig. 1, *(arrow)* the addition of catalase to the cell anti-IgE mixture permitted anti-IgE to stimulate histamine release (50.8%), presumably because catalase, by destruction of H_2O_2, eliminated the inhibitory effect of the high concentration of H_2O_2 (100 μM). This finding indicates that H_2O_2 did not irreversibly damage the functional reactivity of the basophils. The kinetic relationships of the anti-IgE-stimulated release of histamine, H_2O_2, and O_2^- is illustrated in Fig. 2. The rate of histamine release was faster than the rate of generation of H_2O_2, and the rate of O_2^- generation was the slowest. Basophil histamine release in reponse to H_2O_2 plateaued at 20 minutes (data not presented).

Anti-IgE and opsonized zymosan stimulation of H_2O_2 generation in leukocyte fractions of different composition

In these experiments, H_2O_2 generation by cell suspensions relatively enriched with respect to monocytes, lymphocytes, basophils, neutrophils, or eosinophils was measured after exposure to anti-IgE (20 μg/ml) or opsonized zymosan (1 mg/ml) at a cell concentration of 2.5 × 10^6/ml. As observed in Table I, anti-IgE stimulated the generation of H_2O_2 only in the basophil-containing fraction (2% basophils compared to 0% to 0.03% in the other cell fractions). Opsonized zymosan also stimulated H_2O_2 production

Histamine release (%)	H_2O_2 generation (nmol/min)	Superoxide generation (nmol/min)
3.1 ± 3.1	0.001 ± 0.001	0.026 ± 0.026
12.1 ± 2.1	0.105 ± 0.036	0.035 ± 0.035
15.7 ± 1.1†	0.238 ± 0.047	1.629 ± 0.900
15.5 ± 0.4‡	0.285 ± 0.047	0
9.1 ± 1.8	0.168 ± 0.001	0.562 ± 0.093
11.6 ± 2.2	0.179 ± 0.015	0
10.4 ± 2.4	nd	nd
13.4 ± 5.4	nd	nd
12.5 ± 2.6	nd	nd
8.7 ± 0.4§	nd	nd
14.8 ± 3.0	0.326 ± 0.115	0.069
14.8 ± 2.0	0.306 ± 0.159	2.813

in the basophil-containing fraction and in the monocyte-enriched fraction as well; some increase in the neutrophil and the neutrophil plus eosinophil fractions was also noted. The background value of H_2O_2 formation in the neutrophil and neutrophil plus eosinophil fractions presumably reflects a higher rate of endogenous H_2O_2 synthesis by these cells as compared with the other fractions.

Effect of neutrophil- or eosinophil-derived oxygen derivatives on basophil histamine release

Generation of H_2O_2 and O_2^- and the effect of inhibitory agents. In these experiments we studied the effect of adding neutrophil- or eosinophil-rich cell fractions to the basophil-containing cell suspension, with or without opsonized zymosan, on the generation of H_2O_2 and O_2^-. Each of the cell fractions was obtained by Percoll density gradient centrifugation as described in Methods; 1×10^6 cells of the "basophil fraction" were incubated alone or with 2.5×10^6 cells per milliliter of added neutrophil- or eosinophil-enriched fractions with or without added opsonized zymosan (1 mg/ml) in a final volume of 1.0 ml. Incubation time was 40 minutes at 37° C.

The composition of the various cell fractions in these experiments was as follows: *basophil fraction:* basophils, 3.1 ± 1.1%; monocytes, 3.0 ± 0.5%; lymphocytes, 93.8 ± 1.6%; and neutrophils, 0.1 ± 0.05%; *neutrophil fraction:* neutrophils, 98.8 ± 0.3%; lymphocytes, 0.9 ± 0.6%; eosinophils, 0.3 ± 0.3%; *eosinophil fraction:* eosinophils, 89.0 ± 7.6%; neutrophils, 9.8 ± 6.4%; and lymphocytes, 1.2 ± 1.2%.

As observed in Table II, control release of histamine induced by opsonized zymosan was 3.1 ± 3.1%, and this was associated with low levels of H_2O_2 formation and O_2^- generation. In the presence of neutrophils, histamine release increased to 12.1 ± 2.1% in conjunction with a 100-fold increase in the rate of H_2O_2 generation but a negligible change in O_2^- generation. However, when the neutrophil fraction plus opsonized zymosan were added together to the "basophil fraction," histamine release increased significantly to 15.7 ± 1.1% ($p < 0.05$) associated with a 2.3-fold increase in H_2O_2 and a 47-fold increase in the amount of O_2^- generated. There was a significant correlation between H_2O_2 formation and histamine release ($r = 0.784$; $p < 0.01$).

In order to determine which oxygen derivative was responsible for histamine release, the effect of several agents that degrade or scavenge oxygen derivatives was examined. Table II illustrates that the addition of 50 μg/ml of SOD, which dismutates O_2^- to H_2O_2[27, 28] did not alter basophil histamine release stimulated by the neutrophil-opsonized zymosan mixture, whereas at the same time, it reduced measurable O_2^- to zero and had no effect on the rate of H_2O_2 formation.

Because extracellular H_2O_2 is degraded by catalase,[27, 28] the effect of this enzyme on the histamine release apparently caused by H_2O_2 was examined. When catalase (100 μg/ml) was added to the basophil-neutrophil = opsonized zymosan mixture, the rate of H_2O_2 formation was reduced 73.4 ± 14.0%, and histamine release was decreased by 58.7 ± 15.3%.

Alpha-tocopherol acetate is a scavenger of $^1O_2$ and $OH^\cdot$, and D-mannitol is a scavenger of $OH^\cdot$.[27, 28] The effects of these substances on neutrophil-opsonized zymosan-induced histamine release are presented in Table II where it can be observed that α-tocopherol slightly decreased histamine release, but D-mannitol had no effect. In other experiments, α-tocopherol and D-mannitol had no effect on H_2O_2 generation by neutrophils (data not presented).

When eosinophil-enriched cell fractions were added to the basophil fraction, histamine release of 14.8 ± 3.0% was detected in conjunction with spontaneous unstimulated increase in the rate of formation of H_2O_2 (0.326 ± 0.115 nmol/min). Addition of opsonized zymosan did not alter histamine release or the rate of H_2O_2 formation, at the same time causing a large increase in O_2^- generation.

Finally, to establish the key role of H_2O_2 as the leukocyte (neutrophil) product responsible for histamine release, neutrophils (2.5×10^6 cells per milliliter) were exposed to opsonized zymosan for 40 min-

TABLE IV. Calcium and energy requirements for H_2O_2–induced basophil histamine release*

Additions		
Ca^{2+}	glucose	Histamine release (%)
0	0	8.8 ± 0.7
+	0	9.8 ± 0.9
0	+	9.3 ± 0.4
+	+	21.0 ± 2.8
2-Deoxyglucose (1 mmol/L added to glucose-containing medium)		
+	0	20.0 ± 8.2
+	+	7.9 ± 5.9

*With the basophil-containing leukocyte suspensions used in these experiments, 100 μM of H_2O_2 caused approximately 20% histamine release after 40 minutes; averaged results of two experiments. Note that histamine release of 20.0 ± 8.2% in the presence of glucose is reduced to 7.9 ± 5.9% with the addition of 1 mmol/L of deoxyglucose (averaged results of two experiments).

utes at 37° C, and the supernatant was collected after centrifugation. The supernatant was divided, and one half was treated with catalase (100 μg/ml) for 40 minutes at 37° C. The basophil-containing cell fraction was then added to the untreated and catalase-treated supernatants. From Table III it is obvious that catalase completely destroyed the histamine-releasing activity derived from the opsonized zymosan-treated neutrophils. Thus, H_2O_2 appears to be the trigger that is synthesized by neutrophils and that stimulates histamine release.

Ca^{2+} and energy dependence of H_2O_2-induced basophil histamine release. The requirement of Ca^{2+} ions and an energy source (glucose) for H_2O_2-induced basophil histamine release was studied by incubating basophil-containing leukocyte suspensions (1×10^6 cells per milliliter) in the absence or presence of 1 mmol/L of Ca^{2+} and with or without glucose, 4.4 mmol/L. Table IV illustrates that the presence of both Ca^{2+} ions and glucose was required to support histamine release stimulated by H_2O_2 (100 μM). The requirement for glucose to enable histamine release was borne out by the inhibitory effect of 2-deoxyglucose (1 mmol/L).

Effect of theophylline on H_2O_2-induced basophil histamine release. Theophylline (1 mmol/L) had no effect on basophil histamine release induced by 100 μM of H_2O_2 (Table V), suggesting that phosphodiesterase inhibition had no effect on H_2O_2-induced histamine release.

TABLE V. Effect of theophyllline on H_2O_2–induced basophil histamine release*

Additions	Histamine release (%)
Theophylline (1 mmol/L)	7.0 ± 4.3
H_2O_2 (100 μM)	16.8 ± 9.0
H_2O_2 (100 μM) plus theophylline (1 mmol/L)	18.1 ± 10.9

*Leukocyte suspensions were incubated for 40 minutes; averaged results of four experiments.

Effect of flavonoids on basophil histamine release and H_2O_2 generation stimulated by anti-IgE. As illustrated in Fig. 3, quercetin and apigenin inhibited histamine release and H_2O_2 generation in basophil-containing cell fractions in a concentration-dependent manner (5 to 50 μM). By contrast, taxifolin caused a large inhibition of H_2O_2 formation but had a much smaller effect on histamine release.

DISCUSSION

The present experiments confirm that H_2O_2 stimulates histamine release from human basophils.[17] The experiments also demonstrate that low concentrations (4 μM) of H_2O_2 augment anti-IgE-induced histamine release, whereas high concentrations (100 μM) inhibit histamine release. The reversibility of the inhibitory effect of the high concentration of H_2O_2 was demonstrated by the finding that addition of catalase rendered the basophils normally responsive to the histamine-releasing effect of anti-IgE and also established that 100 μM of H_2O_2 does not impair the functional reactivity of basophils to an immunologic stimulus (Fig. 1). This information together with the kinetic data presented in Fig. 2 demonstrate that anti-IgE-induced basophil histamine release is not H_2O_2 dependent but rather that immunologic basophil activation is also accompanied by the generation of oxygen metabolites.

The addition of anti-IgE to various cell fractions relatively enriched in monocytes, lymphocytes, basophils, neutrophils, or neutrophils plus eosinophils caused the generation of H_2O_2 only in the cell fraction containing the largest number of basophils. This observation demonstrates that immunologic activation of basophils is accompanied by H_2O_2 generation (Table I). Opsonized zymosan also stimulated the formation of H_2O_2 in the basophil fraction, indicating that complement activation of basophils also stimulates H_2O_2 formation. In addition, opsonized zymosan stimulated H_2O_2 formation in the monocyte fraction (Table I), a finding consistent with activation of monocyte phagocytosis. In view of these observations, it appears pos-

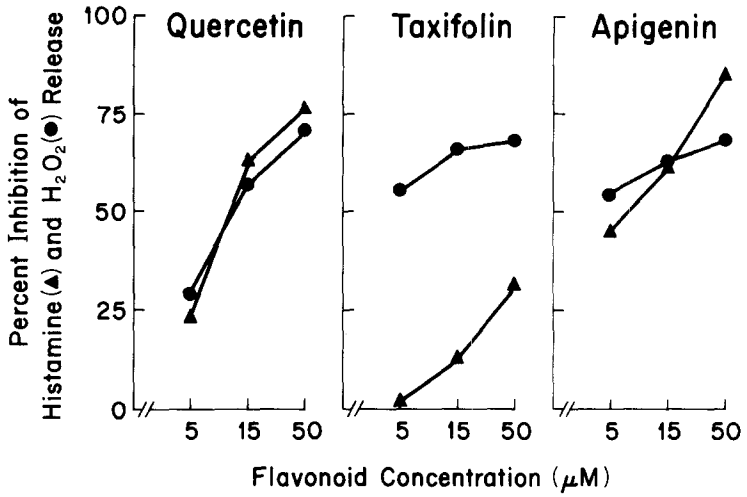

FIG. 3. Inhibitory activity of three flavonoids on human basophil H_2O_2 generation and histamine release stimulated by anti-IgE. Each *point* represents the averaged results of three experiments. The range of control H_2O_2 generation was 0.339 to 1.100 nmol/1 × 10^6 basophil per minute. The average control histamine release was 17.6% to 43.1%. H_2O_2 (●); histamine release (▲).

sible that monocyte-, neutrophil-, and eosinophil-containing (see below) inflammatory loci might generate H_2O_2 that in turn could augment immunologically induced mediator release from basophils and mast cells.

H_2O_2–induced basophil histamine release was found to depend on the presence of extracellular Ca^{2+} ions, to be energy dependent as demonstrated by low histamine release in the absence of buffer glucose, and inhibition of the glucose effect in the presence of 2-deoxyglucose. Also, theophylline failed to inhibit H_2O_2–induced histamine release, suggesting that increased concentrations of cyclic AMP do not inhibit histamine release induced by this substance. This finding is in keeping with the fact that increased cyclic AMP does not inhibit ionophore A23187–induced histamine release or the effect of Sr^{2+} to increase histamine release from human basophils; it is also in agreement with the failure of theophylline to inhibit H_2O_2–induced histamine release from rat mast cells.[17]

The present experiments also demonstrated that incubation of basophil-containing leukocyte suspensions with autologous neutrophil- or eosinophil-enriched cell suspensions together with opsonized zymosan stimulated histamine release. Although it is well recognized that C5a induces basophil histamine release,[29] we found that opsonized zymosan (C5a adsorbed to zymosan) caused negligible histamine release under the conditions of these experiments.

Incubation of neutrophil- or eosinophil-enriched leukocyte fractions with opsonized zymosan stimulated a marked increase in the rate of generation of H_2O_2 and O_2^-. To determine which of these oxygen derivatives was responsible for the increased histamine release noted in experiments in which neutrophil-rich and eosinophil-rich cell suspensions were added to the basophil-containing suspensions, we performed experiments with agents that degrade or scavenge the oxygen metabolites. Histamine release correlated with the rate of H_2O_2 generation and was decreased in the presence of catalase (Table II). Also, the histamine-releasing activity of supernatants of neutrophils treated with opsonized zymosan was completely abolished by treatment of the supernatants with catalase (100 μg/ml). By contrast, SOD strikingly reduced measurable O_2^- without affecting H_2O_2 generation or histamine release; apparently SOD converted all generated O_2^- to H_2O_2. These findings support the concept that it is leukocyte-derived H_2O_2 that is the molecular species responsible for histamine release under the conditions of the present experiments. The nonsignificant effect of D-mannitol and α-tocopherol favors the conclusion that hydroxyl radicals and $^1O_2$ are not involved in stimulated basophil histamine release.

Quercetin and apigenin but not taxifolin inhibited histamine release stimulated by anti-IgE, confirming earlier observations.[23] However, all three flavonoids inhibited the generation of H_2O_2. This finding suggests that H_2O_2 is not required for immunologically stimulated histamine release but represents another aspect of basophil activation. This position is strengthened by the finding that histamine release preceded H_2O_2 generation (Fig. 2). The use of certain flavonoids as

biochemical probes into the mechanism and results of cell activation is also suggested by these experiments.

The differing pharmacologic-biochemical effects of the three flavonoids studied may related to their specific structural features: quercetin (3,5,7,3′,4′pentahydroxyflavone) and apigenin (5,7,4′trihydroxyflavone) are planar molecules, whereas taxifolin (3,5,7,3′4′-pentahydroxyflavanone) (dihydroquercetin) is nonplanar (buckled oxygen-containing heterocyclic C ring).* Thus, the histamine-releasing biochemical step(s) in basophils generated by an immunologic stimulus is (are) sensitive to planar flavonoids, but the biochemical system(s) required for H_2O_2 generation by the same stimulus is (are) sensitive to both planar (quercetin and apigenin) and nonplanar (taxifolin) flavonoids. These structure-activity relationships will be discussed in a future publication.

*Cody V, Middleton E: Unpublished observations.

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