Biochemical Pharmacology, Vol. 29, pp. 741–745.
Pergamon Press Ltd. 1980. Printed in Great Britain.

PROPERTIES OF HYDROGEN PEROXIDE-INDUCED HISTAMINE RELEASE FROM RAT MAST CELLS

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(*Received* 24 *August* 1979; *accepted* 27 *September* 1979)

Abstract—Incubation of rat peritoneal mast cells with hydrogen peroxide results in a marked release of histamine. Maximal release is observed with 0.05–0.1 mM H_2O_2, but higher concentrations of H_2O_2 instead suppresses the release. Histamine release starts after about 2 min of lag time and reaches a plateau in about 10 min. Hydrogen peroxide-induced release does not exceed 50–60 per cent of total histamine if the incubations are prolonged or additional H_2O_2 is given at 10 min. This would be explained by the fact that H_2O_2 causes impairment of the histamine releasing system of mast cells simultaneously with the release of histamine. Hydrogen peroxide-induced release is not due to nonspecific lysis of the cells because lactate dehydrogenase, a cytoplasmic enzyme, is not liberated during the reaction. The reaction requires the presence of Ca^{2+}, is enhanced by D_2O and suppressed by colchicine. It is not, however, affected by dibutyryl cAMP or dibutyryl cGMP. No significant alteration of intracellular levels of cyclic AMP and cyclic GMP is observed during the incubation of mast cells with 0.1 mM H_2O_2. These results indicate that microtubular functions would be involved in the releasing reaction although they are not under the control of cyclic nucleotides. Microscopic observation shows that H_2O_2-induced release is accompanied by degranulation.

We have been working on the toxicities of active species of oxygen against biological systems by employing xanthine oxidase and hypoxanthine as the source of the active species. During the course of this study, we found that active species of oxygen such as superoxide anion (O_2^-), hydroxyl radical ($OH\cdot$) and hydrogen peroxide could cause acute inflammation in rats in which H_2O_2-induced histamine release would play an important role. These results have been published in preliminary forms [1, 2]. It has been reported that active species of oxygen, including H_2O_2, are released from activated phagocytes [3–5]. These active species are implicated to be involved in the generation and development of inflammation [6–10]. Thus, it seems interesting in a physiological sense that H_2O_2 is able to cause a specific biological reaction like histamine release. It is well known that mast cells liberate histamine by various kinds of stimuli such as antigens, compound 48/80, A 23187 (a calcium ionophore), concanavalin A, ATP, etc. [11, 12]. In the present paper, we investigated the properties of H_2O_2-induced histamine release from rat peritoneal mast cells in comparison with those induced by other stimuli. The mechanism of H_2O_2-induced histamine release is discussed.

MATERIALS AND METHODS

Materials. Male rats of Sprague–Dawley strain weighing 300–400 g were used throughout this work. Reagents were obtained from the following sources; DBcAMP, DBcGMP, ATP, catalase and bovine serum albumin from Sigma, colchicine from Inverni & Della Beffa, D_2O from E. Merck, compound 48/80 from Wellcome and Ficoll 400 from Pharmacia. Prostaglandin E_1 was presented by Teijin Limited. Radioimmunoassay kits for cAMP and cGMP were kindly supplied from Yamasa Shoyu company.

Isolation and purification of peritoneal mast cells from rats. Medium A and B with the following compositions were used. Medium A contained 160 mM NaCl, 3 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 7 mM KH_2PO_4, 9 mM Na_2HPO_4, 10 mM glucose and 0.5% bovine serum albumin. In medium B, the bovine serum albumin and glucose were omitted. Rat mast cells were collected by washing the peritoneal cavity twice with 20 ml of cold medium B and were purified by 30% Ficoll 400 gradient centrifugation as described by Cooper and Stanworth [12]. The purified mast cells were suspended in medium A to give about 10^6 cells/ml. When the experiments were performed in Ca^{2+}-free conditions, mast cells were collected by using Ca^{2+}-free medium A and B.

Histamine release studies. The standard reaction mixture for the assay of H_2O_2-induced histamine release consisted of 0.8 ml of medium A, 0.1 ml of 1 mM H_2O_2 and 0.1 ml of mast cell suspension (*ca.* 10^5 cells). The reactions were carried out at 37° for 10 min and terminated by treating with 260 units of catalase for 1 min followed by cooling in an ice bath. Released histamine was determined fluorometrically as described by Shore *et al.* [13]. Catalase treatment was necessary because residual H_2O_2 disturbed fluorometric assay of histamine. In our previous paper [2], we did not perform catalase treatment. Therefore, the maximal release and the concentrations of H_2O_2 which elicit the maximal release were lower than those reported in this paper. In some cases,

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§ Abbreviations: DBcAMP, dibutyryl cyclic AMP; DBcGMP, dibutyryl cyclic GMP; PGE_1, prostaglandin E_1.

0.5 mM ATP or 1 μg/ml compound 48/80 was added in place of H_2O_2.

Radioimmunoassay of cyclic nucleotide levels in mast cells. 5×10^5 rat mast cells were incubated with 0.1 mM H_2O_2 for various times in 0.2 ml medium A. The reactions were stopped by adding 2 ml of cold medium A. After centrifugation at 4° for 2 min, the cell pellets were immediately frozen in dry ice–acetone. The samples were thawed by adding 0.2 ml of acetone under vortexing. Acetone was removed on a water bath at 80° and the residue was dissolved in 0.6 ml of water. A 0.5 ml portion was lyophilized and redissolved in 50 μl of water, which was directly used for radioimmunoassay. The measurable range of this radioimmunoassay was about 10–3000 fmoles for both cAMP and cGMP. The principle of this procedure has been described by Honma *et al.* [14].

Microscopic observation of mast cells. Mast cells were incubated with 0.1 mM H_2O_2 for 10 min as described above and were fixed with 4% formaldehyde. Morphological observation was made by employing phase contrast microscope.

Assay of lactate dehydrogenase activity in mast cells. Lactate dehydrogenase activities were assayed by NADH-oxidation in the presence of pyruvate as described by Kornberg [15].

In each table and figure, only typical results obtained from repeated experiments are presented.

RESULTS

H_2O_2-induced histamine release. As shown in Fig. 1, the incubation of isolated rat peritoneal mast cells with H_2O_2 resulted in a marked release of histamine. Maximal release was observed with 0.05–0.1 mM H_2O_2, but higher concentrations of H_2O_2 rather decreased the release.

Figure 2 illustrates the kinetics of histamine release induced by 0.1 mM H_2O_2. The histamine release began after 2 min and reached plateau in about 10 min. The prolonged incubation or addition of extra H_2O_2 (0.05 mM) at 10 min did not liberate further histamine.

Impairment of histamine releasing system during incubation with H_2O_2. As stated in the previous section, H_2O_2-induced histamine release did not exceed 50–60 per cent at the highest. The following experiments were performed to investigate this question. When Ca^{2+} was omitted from medium A, H_2O_2-induced histamine release was lowered to about a half of that obtained under Ca^{2+}-supplemented conditions. In further experiments the reaction was started with H_2O_2 alone, Ca^{2+} was added at various times, and the incubation was continued for a further 10 min. As shown in Fig. 3, the final histamine release gradually decreased as the time before Ca^{2+}-addition was increased. When Ca^{2+} was added after 10 min, no enhancement of the release was observed. These facts suggest that the incubation of mast cells with H_2O_2 results in an impairment of histamine releasing system simultaneously with the release of histamine. Figure 3 also shows that, when catalase was added with Ca^{2+}, further histamine release was completely abolished. As illustrated in Fig. 4a, a time-dependent suppression of compound 48/80-induced histamine release was observed when the mast cells were incubated with 0.1 mM H_2O_2. Hydrogen peroxide,

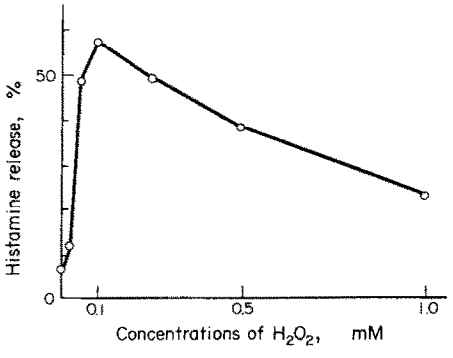

Fig. 1. Effect of H_2O_2 concentrations on histamine release from rat mast cells. Rat mast cells (*ca.* 10^5 cells) were incubated with varying concentrations of H_2O_2 at 37° for 10 min as described in Materials and Methods.

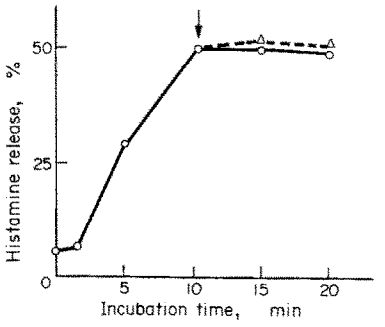

Fig. 2. Kinetics of H_2O_2-induced histamine release from rat mast cells. Mast cells (*ca.* 10^5 cells) were incubated at 37° for the time indicated in the presence of 0.1 mM H_2O_2. At 10 min (indicated by an arrow), 0.05 mM H_2O_2 was added and further incubated for 10 min (– –△– –).

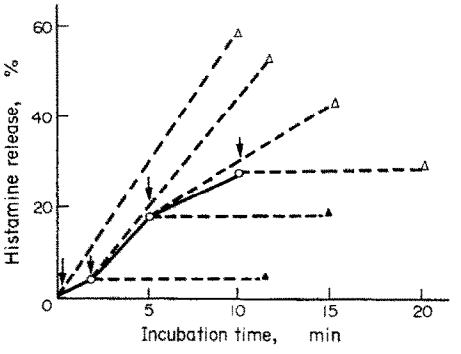

Fig. 3. Loss of Ca^{2+}-dependent enhancement of H_2O_2-induced histamine release during incubation of rat mast cells with 0.1 mM H_2O_2. Mast cells (*ca.* 10^5 cells) were incubated at 37° with 0.1 mM H_2O_2 in Ca^{2+}-free medium A. At the time indicated by an arrow, 1 mM $CaCl_2$ or catalase in addition to 1 mM $CaCl_2$ was added. The incubation was continued further for 10 min. (—○—) Release by H_2O_2 in Ca^{2+}-free medium. (– –△– –) Release after addition of 1 mM $CaCl_2$. (– –▲– –) Release after addition of 1 mM $CaCl_2$ and catalase (260 units).

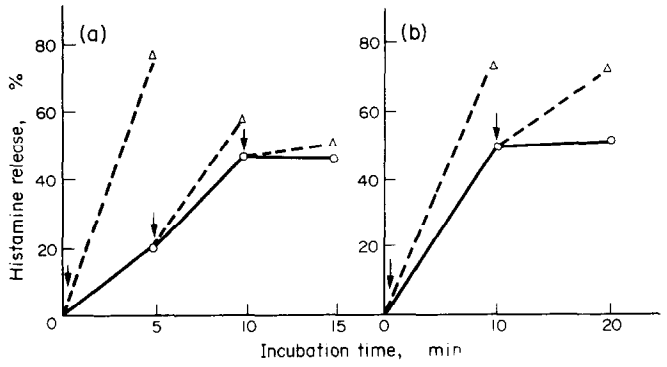

Fig. 4. Effect of incubation of rat mast cells with H_2O on histamine release induced by compound 48/80 or ATP. Mast cells (*ca.* 10^5 cells) were incubated with 0.1 mM H_2O_2 at 37° for various times. Panel a: Compound 48/80 (1 μg/ml) and catalase (260 units) were added at the time indicated by an arrow and incubation continued for 5 min. (—○—) H_2O_2-induced release, (- -△- -) Release after addition of compound 48/80 and catalase. Panel b: 0.5 mM ATP was added at 10 min and incubation continued for 10 min. (—○—) H_2O_2-induced release. (- -△- -) Release after addition of 0.5 mM ATP.

however, did not suppress uniformly the histamine release by various stimuli since 0.1 mM H_2O_2 had no effect on ATP-induced histamine release, as shown in Fig. 4b. However, much higher concentrations of H_2O_2 (1 mM) did impair ATP-induced release completely (data not shown). This competition between the stimulation and inactivation of the releasing system would explain why H_2O_2-induced release does not exceed a constant value.

Lack of lactate dehydrogenase release during H_2O_2*-induced histamine release.* Hydrogen peroxide is a powerful oxidizing agent and might cause nonspecific damage in the cell membrane. The release of lactate dehydrogenase, a cytoplasmic enzyme, was measured in order to investigate whether H_2O_2-induced release is due to nonspecific lysis of cell membrane. Table 1 demonstrates that lactate dehydrogenase was not liberated by H_2O_2, thus indicating that nonspecific lysis of the cell was not responsible for the histamine release.

Effect of colchicine and D_2O. In order to investigate the mechanism of H_2O_2-induced histamine release, the effects of various agents that are known to suppress or enhance antigen-induced histamine release were studied. As indicated in Table 2, colchicine markedly suppressed the release, but D_2O enhanced it to a considerable extent. It is well known that the former agent suppresses; in contrast the latter stimulates the function of microtubules [16].

Effect of cyclic nucleotides or PGE_1. The effect of cyclic nucleotides which are thought to regulate antigen-induced histamine release through the control of microtubular assembly [17] were investigated. As shown in Table 3, neither DBcAMP nor DBcGMP affected the release. PGE_1, which is known to elevate cAMP in mast cells [18], had no effect on H_2O_2-induced release, while DBcAMP or PGE_1 inhibited compound 48/80-induced release significantly.

Alteration of intracellular cyclic nucleotide levels by H_2O_2. When mast cells were incubated with 0.1 mM H_2O_2 for various times, the alteration of intracellular cyclic nucleotide levels was examined. As depicted in Fig. 5, no appreciable changes of cAMP and cGMP levels were observed, thus suggesting that H_2O_2-induced histamine release occurs by by-passing cyclic nucleotide-dependent stages.

Morphological changes of mast cells during incubation with H_2O_2. It is well known that histamine release by various stimuli such as antigens or compound 48/80 is accompanied by degranulation [19, 20]. It was, therefore, investigated whether

Table 1. Release of histamine and lactate dehydrogenase from rat mast cells incubated with H_2O_2*

Incubation conditions	Release (%)	
	Histamine	Lactate dehydrogenase
None	5.1	2.0
+ H_2O_2 (0.1 mM)	44.3	1.0

* 5×10^5 mast cells were incubated in 1 ml of medium A at 37° for 10 min in the presence or absence of 0.1 mM H_2O_2. After separation of supernatants and cells by centrifugation at 4°, cell pellets were subject to three cycles of freezing and thawing in 1 ml of medium A for extracting intracellular enzymes. Lactate dehydrogenase activities in the cells and supernatants were assayed as described in Materials and Methods.

Table 2. Effect of D_2O or colchicine on H_2O_2-induced histamine release from rat mast cells*

Additions		Histamine release (%)
None		44
D_2O	10%	48
	20%	60
	30%	69
Colchicine	0.2 mM	38
	0.5 mM	26
	1.0 mM	17

* Rat mast cells (*ca.* 10^5 cells) were incubated with 0.1 mM H_2O_2 in the presence of the indicated concentrations of D_2O or colchicine at 37° for 10 min.

Table 3. Effect of DBcAMP, DBcGMP or PGE_1 on H_2O_2-induced histamine release from rat mast cells

Additions		Histamine release (%)	
		H_2O_2	compound 48/80
Expt. (1)	none	50	79
	DBcAMP (1 mM)	56	49
	PGE_1 (25 μM)	51	53
Expt. (2)	none	47	82
	DBcGMP (1 mM)	47	90

* Rat mast cells (*ca.* 10^5 cells) were preincubated at 37° with the indicated amounts of DBcAMP, DBcGMP or PGE_1 for 10 min. Then 0.1 mM H_2O_2 or 1 μg/ml compound 48/80 was added and the incubation was continued for 10 min.

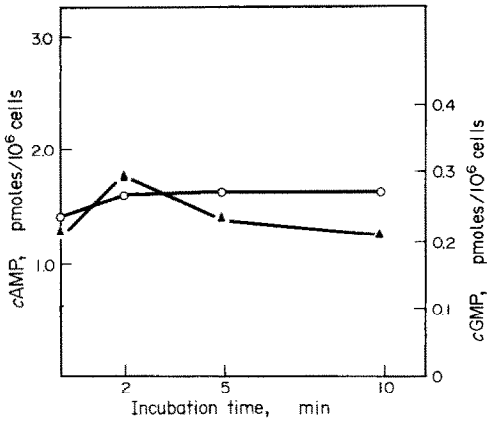

Fig. 5. Alteration of the level of cAMP or cGMP during incubation of rat mast cells with H_2O_2. 5×10^5 mast cells were incubated at 37° with 0.1 mM H_2O_2 for the time indicated. Intracellular cyclic nucleotide levels were assayed as described in Materials and Methods. (○) cAMP, (▲) cGMP.

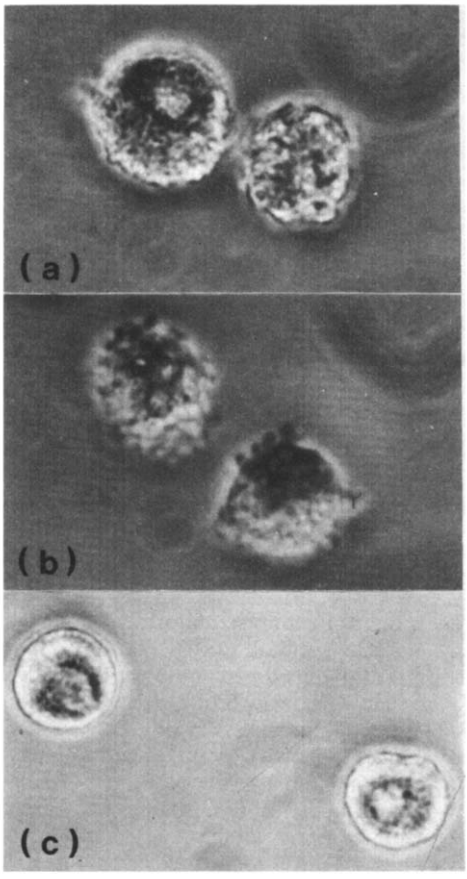

Fig. 6. Morphological changes of rat mast cells after incubation with H_2O_2 or compound 48/80. Mast cells were incubated at 37° with (a) 0.1 mM H_2O_2 for 10 min or (b) 1 μg/ml compound 48/80 for 5 min. (c) Control cells. Magnification was 720 fold.

degranulation would be observed in the case of H_2O_2-induced release. As shown in Fig. 6, degranulation did occur with 0.1 mM H_2O_2, although to a lesser extent as compared with compound 48/80. In the case of 1 mM H_2O_2, which released less histamine than 0.1 mM H_2O_2, only weak degranulation was observed (not shown in this figure).

DISCUSSION

Histamine is released from mast cells by various stimuli other than antigens, such as compound 48/80, ATP, concanavalin A or A 23187 [11, 12]. Our data described above indicate that H_2O_2 possesses the ability to release histamine without causing nonspecific lysis of mast cells. One characteristic feature of H_2O_2-induced histamine release is that the release did not exceed 50–60 per cent of total histamine. As depicted in Figs. 2–4, it was demonstrated that the incubation of mast cells with 0.1 mM H_2O_2 resulted in the impairment of the histamine releasing mechanism as well as in the release of histamine. The fact that higher concentrations of H_2O_2 (1 mM) suppressed the release (Fig. 1) would be explained by assuming that the impairment proceeds more readily than the releasing reaction. Catalase, when added

at any time during the incubation, terminated further release of histamine completely (Fig. 3). This implies that extracellular H_2O_2, but not that which penetrated the cell, would be responsible for the induction of histamine release and that it may act on the membrane of the mast cells. The impairing effect of 0.1 mM H_2O_2 is not nonspecific but would appear to be directed to specific stages of histamine release because it suppressed compound 48/80-induced release, but not that induced by ATP. On the other hand, 1 mM H_2O_2 blocked ATP-induced release. Therefore, this concentration of H_2O_2 appears to be nonspecific. The suppression of compound 48/80-induced release was observed after removal of residual H_2O_2 by catalase treatment, thus indicating that the suppressing effect appears to be irreversible.

The effects of colchicine or D_2O, requirement of energy sources [2] and microscopic observation of degranulation suggest that microtubules are involved in H_2O_2-induced release. In antigen- or compound 48/80-induced release from mast cells, the microtubular functions are regulated by cyclic nucleotide levels [16, 17]. That is, cAMP inhibits the assembly of microtubules and leads to the suppression of histamine release. On the other hand, cGMP has opposite effects. H_2O_2-induced histamine release, however, was not affected by DBcAMP or DBcGMP. Moreover, there was no significant alteration of intracellular cyclic nucleotide levels when mast cells were incubated with H_2O_2. It has been reported that a transient alteration of cAMP level was observed in mast cells incubated with various stimuli [21, 22]. Thus, it appears that H_2O_2-induced histamine release occurs by the stimulation of the microtubular functions via a cyclic nucleotide-independent route. This resembles the case of A 23187-induced histamine release from human basophils [23].

Recently, it has been reported that mast cell granules contain peroxidase activity [24]. This peroxidase activity may be involved in H_2O_2-induced histamine release. But in our preliminary experiments, aminotriazole, an inhibitor of peroxidase, had no effect on H_2O_2-induced release. H_2O_2-induced histamine release was observed not only in rat mast cells but also in human peripheral blood leukocytes. We found that 0.2–0.5 mM H_2O_2 released 20–40 per cent histamine from human leukocyte fraction containing basophils (unpublished data).

It has been suggested that O_2^- and H_2O_2 liberated from activated macrophages or neutrophils would be responsible for the bactericidal action, tissue damage or cytotoxicity [3, 7, 25]. Although the physiological significance of our observation has not been clarified *in vivo*, these data imply that active species of oxygen, like H_2O_2, could modify inflammatory processes or other pathological states by inducing a specific physiological reaction as described above.

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