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SHORT COMMUNICATIONS

Xanthine oxidase-induced histamine release from isolated rat peritoneal mast cells: involvement of hydrogen peroxide

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The generation of oxygen radicals in biological systems and their toxicities have been described by many authors [1–5]. There are several lines of evidence suggesting that active species of oxygen like superoxide anion (O_2^-) or hydroxyl radical ($OH^\cdot$) may be involved in inflammatory processes [6–8]. We have reported that the injection of xanthine oxidase and hypoxanthine (XOD–HPX) into the foot paw of rats resulted in an acute foot-edema. Active species of oxygen like O_2^-, H_2O_2 and $OH^\cdot$ derived from XOD reaction are considered to be involved in the paw swelling [9]. The observation that diphenhydramine strongly inhibited the foot-edema suggests that histamine might play an important role as a mediator in this inflammation. In order to clarify the mechanism of XOD–HPX-induced foot-edema, we investigated whether XOD–HPX could cause histamine release from rat mast cells.

XOD oxidizes xanthine or hypoxanthine to uric acid and concomitantly evolves O_2^- which is then converted to H_2O_2, $OH^\cdot$ and singlet oxygen ($^1O_2$) according to the following equations [3, 10].

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2. \quad (1)$$

$$O_2^- + H_2O_2 + H^+ \rightarrow H_2O + OH^\cdot + {}^1O_2. \quad (2)$$

Singlet oxygen, however, may be readily scavenged by hypoxanthine, xanthine and uric acid as reported by Kellogg and Fridovich [10]. XOD–HPX is, therefore, an excellent source of O_2^-, H_2O_2 and $OH^\cdot$. In the present paper, we report that hydrogen peroxide produced by XOD–HPX released histamine from isolated rat mast cells without causing nonspecific lysis of the cells.

Medium A and B with the following compositions were used throughout this work. Medium A contained 0.16 M 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, bovine serum albumin and glucose were omitted from medium A. Male rats of Sprague–Dawley strain weighing 350–400 g were used. Peritoneal mast cells were collected by lavage of peritoneal cavity with 20 ml of cold medium B, and were purified by Ficoll gradient centrifugation according to the procedure of Cooper and Stanworth [11]. The resulting mast cell suspension in medium A contained about 10^6 cells/ml which corresponded to approximately 10 μg/ml of histamine. Complete system for the assay of histamine release from rat mast cells induced by XOD–HPX consisted of 0.7 ml of medium A, 0.1 ml of XOD (50 μg), 0.1 ml of 5 mM hypoxanthine and 0.1 ml of mast cell suspension (*ca.* 10^5 cells). XOD (Boehringer Mannheim, 0.4 u/mg protein) was previously passed through Sephadex G-25 column (1 × 25 cm) equilibrated with 70 mM phosphate buffer (pH 7.0) to remove EDTA and $[NH_4]_2SO_4$. In some experiments, XOD–HPX was replaced by an appropriate amount of H_2O_2. The reactions were carried out at 37° for 10 min and terminated by cooling in an ice-bath. After removing mast cells by centrifugation, released histamine in the supernatant was determined fluorimetrically according to the method of Shore *et al.* [12]. Histamine release was expressed as the per cent of total histamine. All the data were presented as a mean value of duplicate experiments.

Incubation of XOD–HPX with rat peritoneal mast cells resulted in a marked release of histamine as indicated in Table 1. The histamine release was reduced when either XOD or hypoxanthine was omitted, or when boiled XOD was used. Thus, it was indicated that the histamine release was dependent on the enzymic activity of XOD. The reaction products of XOD–HPX, namely, xanthine or uric acid did not cause histamine release. It, therefore, was expected that O_2^- evolved by XOD–HPX, or active species of oxygen derived from O_2^- according to equation (1) and (2) would be involved in the release of histamine.

Table 1. XOD–HPX-induced histamine release from rat mast cells: cofactor requirements and effect of SOD, catalase and D-mannitol

Reaction system	Histamine release (%)	
Experiment 1.		
Complete system	40.0	(100)
−hypoxanthine	14.1	(37)
−XOD	1.4	(4)
Complete system (boiled XOD)	5.6	(14)
Experiment 2.		
Complete system	43.0	(100)
+SOD (50 μg)	43.6	(101)
+catalase (50 μg)	19.8	(46)
+SOD (50 μg) + catalase (50 μg)	18.6	(43)
+D-mannitol (1 mM)	41.8	(97)

In experiment 1, indicated cofactors were omitted from the complete system described in the text. In experiment 2, SOD (Sigma) catalase (Boehringer Mannheim) or D-mannitol was added to the complete system.

In order to study which active species are really responsible for the releasing reaction, effects of superoxide dismutase (SOD), catalase and D-mannitol on XOD HPX-induced histamine release were examined. As shown in Table 1, the histamine release was inhibited markedly by catalase, whereas SOD or D-mannitol had no effects. Combination of SOD with catalase did not enhance the effect of catalase. SOD catalyzes disproportionation of O_2^- into H_2O_2 and O_2, while D-mannitol is a typical scavenger of $OH^\cdot$. These results suggest that H_2O_2 derived from O_2^- according to equation (1) would play a major role in the histamine release from the mast cells. This was further confirmed by the result that histamine was similarly released when the mast cells were incubated with H_2O_2 as illustrated in Fig. 1. Maximal release was observed with 0.05 mM H_2O_2, but higher concentrations of the stimulus were inhibitory. This is presumably due to the loss of viability of the mast cells.

This histamine release induced by XOD–HPX or by H_2O_2 is not due to nonspecific lysis of the mast cells because these releasing stimuli did not liberate lactate dehydrogenase, a cytoplasmic enzyme [11] during histamine release (data not shown).

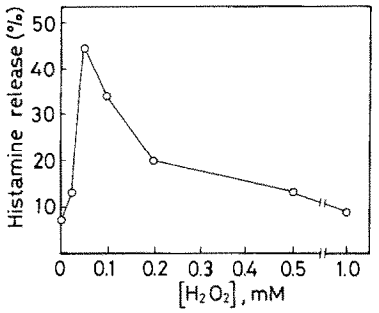

Fig. 1. Effect of H_2O_2 concentrations on histamine release from rat mast cells. The mast cells were incubated with indicated concentrations of H_2O_2 at 37° for 10 min as described in the text.

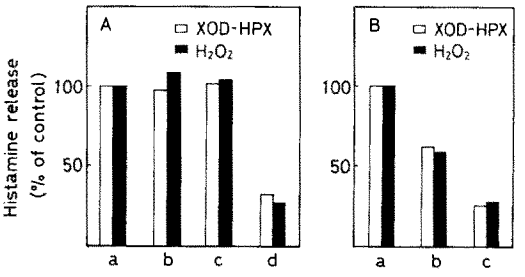

Fig. 2A. Effect of dibutyryl cAMP, prostaglandin E_1 and colchicine on histamine release from rat mast cells induced by XOD–HPX or by H_2O_2. The mast cells were incubated with XOD–HPX or with H_2O_2 (0.05 mM) as described in the text in the presence of the indicated agents. Histamine release was expressed as the per cent of control. (a) control (no addition), (b) +dibutyryl cAMP (1 mM), (c) +prostaglandin E_1 (0.05 mM), (d) +colchicine (1 mM). B. Dependence of histamine release from rat mast cells induced by XOD–HPX or by H_2O_2 upon Ca^{2+} and glucose. Mast cells were prepared in medium A in which $CaCl_2$ and glucose were omitted. Where indicated, $CaCl_2$ or glucose was omitted from the complete system for the assay of histamine release (see text). (a) complete system, (b) minus glucose (8 mM), (c) minus $CaCl_2$ (0.72 mM).

Effects of various agents that are known to inhibit histamine release induced by antigens or by compound 48/80 were studied in order to examine the properties of histamine release induced by XOD–HPX or by H_2O_2. Neither dibutyryl cAMP nor prostaglandin E_1 affected the release (Fig. 2A). Colchicine, however, strongly inhibited the release induced by XOD–HPX as well as that by H_2O_2. Both XOD–HPX- and H_2O_2-induced histamine release were found to be dependent on Ca^{2+} and glucose in a similar manner as shown in Fig. 2B.

The results described above showed that H_2O_2 was a real stimulus in the histamine release induced by XOD–HPX. These results and our previous observation that diphenhydramine strongly suppressed the foot-edema induced by XOD–HPX appear to support the idea that H_2O_2-induced histamine release may play a vital role in causing the paw swelling. Histamine is not, however, a sole mediator in this inflammation because the injection of H_2O_2 or histamine alone did not cause a significant edema. XOD–HPX-induced foot-edema would be a more complicated process in which O_2^-, H_2O_2 and $OH^\cdot$ may act in cooperation not only upon mast cells but also upon other tissue cells, thus leading to the edema.

It has been reported that histamine can be released from mast cells by various stimuli such as antigens, ATP, compound 48/80 or A 23187 (a calcium ionophore) [11]. Histamine release induced by antigens or compound 48/80 is known to be inhibited by theophylline, prostaglandin E_1, dibutyryl cAMP or colchicine [13–15]. On the other hand, A 23187-induced histamine release was suppressed by colchicine and deoxyglucose, but not by dibutyryl cAMP [15]. Lichtenstein proposed that the process of histamine release induced by antigen could be divided into two stages: the first stage that is associated with cAMP and is Ca^{2+}-independent, and the second stage in which histamine release occurs depending on Ca^{2+} and energy sources [15]. He stated that A 23187-induced histamine release "short-circuits" the cAMP-associated stage but has a similar mechanism to the second stage of antigen-induced histamine release. The data in Figs. 2A and 2B indicate that H_2O_2-induced histamine release resembles that induced by A 23187. It remains to be elucidated whether H_2O_2 directly functions as a releasing stimulus. It is possible to postulate that reaction of H_2O_2 with some cellular components (e.g. peroxidation) may produce some changes leading to histamine release.

It has been generally considered that the toxicities of active species of oxygen may be due to their nonspecific attack upon cellular components such as lipids, nucleic acids and proteins [5, 8, 10]. Our results present the possibilities that relatively lower concentrations of active species of oxygen could cause a biological disorder by inducing specific physiological reaction like histamine release.

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