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Activation of Parietal Cell by Mercaptomethylimidazole

A NOVEL INDUCER OF GASTRIC ACID SECRETION

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ABSTRACT. Mercaptomethylimidazole (2-Mercapto-1-methylimidazole, MMI), an antithyroid drug of thionamide group, significantly activated the parietal cell for acid secretion, as evidenced by increased O_2 consumption by more than 2.5-fold over the basal level. When compared, MMI-induced activation was similar to that of histamine but significantly higher than that of isobutylmethylxanthine or carbachol. Activation by MMI was not prevented by receptor blockers of the parietal cell, indicating that its effect was not mediated through the cell surface histamine-H_2 receptor or the muscarinic receptor. However, the activation was almost completely blocked only by omeprazole, an established inhibitor of the terminal proton-pumping H^+-K^+-ATPase of the parietal cell. That MMI-induced activation was coupled with the H^+ transport was further confirmed by significant increase in [^{14}C]-aminopyrine uptake by MMI in rabbit gastric gland preparation. MMI-dependent activation of the parietal cell correlated well with the inhibition of the endogenous peroxidase activity. In vitro studies indicated that MMI irreversibly inactivated both peroxidase and catalase activity of the parietal cell in presence of H_2O_2. As inactivation of these H_2O_2-scavenging enzymes should increase accumulation of intracellular H_2O_2, the effect of latter was studied on acid secretion. H_2O_2 at a low concentration, stimulated acid secretion by sevenfold in isolated gastric mucosa, which was sensitive to omeprazole. It also significantly stimulated [^{14}C]-aminopyrine uptake in gastric gland preparation. We suggest that MMI activated parietal cells to stimulate acid secretion by endogenous accumulation of H_2O_2 through inactivation of the peroxidase–catalase system. BIOCHEM PHARMACOL **54**;2:241–248, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Mercaptomethylimidazole and parietal cell activation; hydrogen peroxide and acid secretion; mercaptomethylimidazole and parietal cell peroxidase-catalase system; mercaptomethylimidazole and gastric acid secretion

Gastric acid secretion is stimulated by several physiological secretagogues such as histamine, acetylcholine, and gastrin through the involvement of second messengers such as c-AMP, Ca^{2+}, or inositol trisphosphate [1–4]. These messengers activate various cellular protein kinases [5, 6] finally resulting in the stimulation of the proton-pumping H^+-K^+-ATPase located in the apical membrane of the parietal cell [7, 8]. This enzyme catalyses the electroneutral exchange of H^+/K^+ involved in the terminal step of HCl secretion [8]. Mercaptomethylimidazole (MMI),† a well-known antithyroid drug of thionamide group [9] that inhibits thyroid peroxidase and lactoperoxidase [10–13], also induces gastric acid secretion [14]. We have reported earlier that gastric mucosa contains a highly active peroxidase [15], mainly located in the parietal cell [16]. MMI-induced acid secretion was found to be correlated with the inhibition of this gastric peroxidase [16]. Further studies indicated that MMI inactivates the purified gastric peroxidase (GPO) as a suicidal substrate in the presence of H_2O_2 [17]. We have also reported earlier that MMI-induced acid secretion is partially sensitive to cimetidine, the H_2-receptor blocker, whereas it is almost completely blocked by omeprazole, an established inhibitor of H^+-K^+-ATPase [14]. However, the mechanism of MMI-induced acid secretion at the cellular level is still not clear today. The present study indicates that MMI stimulates [^{14}C]-aminopyrine uptake as a measure of acid secretion in gastric gland preparation. It also activates the parietal cell to stimulate O_2 consumption, correlating well with the inhibition of the cellular peroxidase activity. Evidence is also presented to show that MMI can directly inactivate cellular peroxidase and catalase activity in the presence of H_2O_2. Moreover, gastric gland, when incubated with low concentration of H_2O_2, stimulates acid secretion, as measured by [^{14}C]-aminopyrine

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† *Abbreviations*: AP, aminopyrine; HBSS, Hank's balanced salt solution; IBMX, isobutylmethyl xanthine; GSH, reduced glutathione; MMI, mercaptomethylimidazole; PTU, propylthiouracil; and TCA, trichloroacetic acid.

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uptake. Results indicate that MMI activates the parietal cell to induce acid secretion through intracellular increase in H_2O_2 by inactivating the cellular peroxidase–catalase system.

MATERIALS AND METHODS

Materials

Protease (Pronase E 4.7 U/mg), collagenase type 1 (295 U/mg), trypsin inhibitor, histamine, carbachol, isobutylmethylxanthine (IBMX), bovine serum albumin (BSA), ethylene-bis-glycol tetraacetic acid (EGTA), mercaptomethylimidazole (MMI), propylthiouracil (PTU), propanolol, nifedipine, and cimetidine were obtained from Sigma, St. Louis, MO. Na[^{125}I] was obtained from the Bhabha Atomic Research Center, Trombay, India. Dimethyl [^{14}C]-aminopyrine [(^{14}C AP), 96 mCi/mmol] and Percoll were obtained from Flurochem, UK, and Pharmacia, Uppsala, Sweden, respectively. Hank's balanced salt solution (HBSS) was prepared according to the instructions given in the GIBCO manual. Omeprazole was a gift from Dr. W. Beil of Medizinische Hochschule, Hannover, Germany.

Methods

ISOLATION OF PARIETAL CELLS. Parietal cells from rat gastric mucosa were isolated as described before [16] by modifications of methods described earlier [18]. In brief, fundic mucosa of three nonfasted rats were rinsed in chilled HBSS pH 7.4, minced quickly, and transferred to a double-layered glass chamber (for water circulation at constant temperature) containing 20 mL of HBSS with 1 mg of protease/mL. It was digested for 15 min at 37°C with continuous gassing with O_2/CO_2 (95:5) over a magnetic stirrer. The released cell suspension was decanted through a coarse nylon mesh and discarded. The remaining tissue was digested as above for another 60 min at 37°C in 20 mL of HBSS containing 0.1% BSA, 1 mM EGTA, 0.2 mg of trypsin inhibitor/mL, and 0.5 mg of collagenase/mL. The cell suspension was passed through the nylon mesh, washed three times in HBSS, and suspended in oxygenated HBSS containing 0.1% BSA but without Ca^{2+}. Cells (10^7) of this fraction were layered over a discontinuous gradient formed with 3 mL each of 10, 20, and 30% (v/v) Percoll in HBSS, keeping lesser concentration at the top of the gradient in a DuPont 15 mL centrifuge tube. The gradient was run at 1075 × *g* for 10 min at room temperature in a Sorval RC-5B centrifuge. Parietal cells were enriched at 10–20% interface, as judged by microscopic observation. These cells were collected, washed three times in HBSS to remove adhering Percoll, and finally suspended in HBSS for further studies.

O_2 CONSUMPTION IN ISOLATED PARIETAL CELL. O_2 consumption of the cells were studied according to the method of Berglindh [19]. After 30 min of incubation at 37°C, 10^6 cells were added to the oxygraph (Gilson) flask containing HBSS with BSA (0.1%) and Ca^{2+} (1 mM) in a final volume of 2 mL. O_2 consumption was measured in presence or absence of desired agent as described in the legend of each table and figure. O_2 consumption was expressed as nmols of O_2 consumed per min per 10^6 cells.

ASSAY OF PEROXIDASE ACTIVITY BY [^{125}I] ORGANIFICATION IN ISOLATED PARIETAL CELL. For peroxidase–catalyzed radioiodide organification *in vitro* by isolated cells, 2×10^5 cells were added in triplicate to a 10 mL scintillation vial containing [^{125}I] (10^6 CPM) and the test drug in a final volume of 1 mL of HBSS pH 7.4 with Ca^{2+} (1 mM) and BSA (0.1%). Cells were incubated at 37°C with shaking (120 rev/min) for 30 min and 0.9 mL of the cell suspension was transferred to microfuge tube. Cells were pelleted by centrifugation (15000 × *g*), lysed with 0.5 mL of distilled water, and TCA was added (final concentration 5%) to precipitate the protein (BSA, 1 mg/mL was added to help precipitation, if necessary). After centrifugation (15000 × *g*), the TCA precipitate was washed with cold 5% TCA containing 1 mM MMI (blocker of peroxidase activity) and 1 mM KI and the protein-bound iodide (radioiodide organified by the cell peroxidase) was counted for radioactivity in a gamma-counter. [^{125}I]organification was expressed as CPM/10^6 cell per 30 min incubation.

ASSAY OF CATALASE AND PEROXIDASE ACTIVITY OF THE PARIETAL CELL. Catalase activity was measured using cell-free homogenate according to the method of Goldstein [20], by following O_2 evolution from H_2O_2 in a Gilson oxygraph. The assay system contained in a final volume of 2 mL; 50 mM sodium phosphate buffer pH 7.2 and a suitable amount of enzyme preparation. H_2O_2 (100 μM) was added last to start the reaction. Results were expressed as nmols of O_2 evolved per min per mg of protein. Peroxidase activity of the cell-free homogenate was measured by I_3^- assay as described earlier [15, 16].

IN VITRO ACID SECRETION BY GASTRIC MUCOSA. For measurement of acid secretion *in vitro*, a chambered gastric mucosal preparation was used as described by Reeves and Stables [21] with slight modifications [16].

IN VITRO ACID SECRETION IN ISOLATED GASTRIC GLAND PREPARATION BY [^{14}C]-AMINOPYRINE UPTAKE. Gastric glands from rabbit gastric mucosa were prepared by enzymatic digestion as previously described [22]. In brief, gastric mucosa was scraped out with the help of a scalpel and chopped finely with scissors. Digestion was performed at 37°C, using 20 mg collagenase/50 mL incubation medium containing NaCl, 140 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $CaCl_2 \cdot 2H_2O$, 1 mM; Hepes, 10 mM; KOH, 5.4 mM; cimetidine, 100 μM; D(+) glucose, 0.5 mg/mL, and BSA, 2 mg/mL. After 40 min, when the digestion was found to be complete by microscopic evaluation, the glands were washed three times with incubation medium without cimetidine and allowed to settle. The medium was removed and

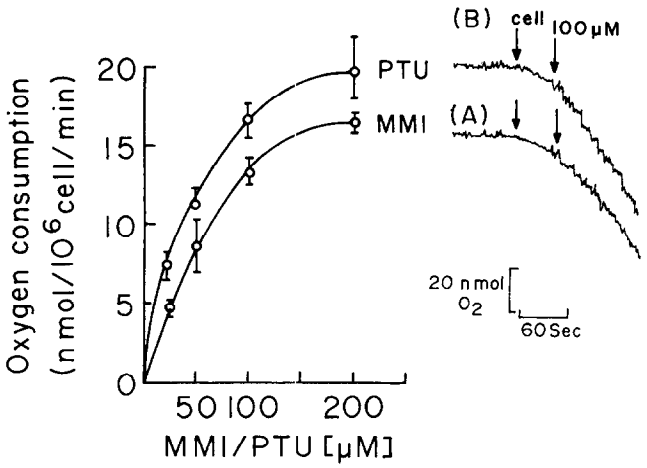

FIG. 1. Effect of MMI or PTU on O_2 consumption by isolated parietal cell: O_2 consumption was measured at 25°C in presence of various concentration of MMI or PTU as indicated. The values were plotted after correction for basal consumption. Inset shows the original oxygraphic tracing at 100 μM MMI or PTU. (A) MMI (B) PTU. (n = 6).

glands were resuspended in incubation medium having no cimetidine. [^{14}C]-aminopyrine uptake was determined in stimulated and unstimulated glands as described [23]. During incubation with [^{14}C]-AP, an aliquot (0.9 mL) was removed at various time interval, centrifuged, and the supernatant was discarded. The pellet was blotted to dryness and dissolved in 0.3 mL of 3 N KOH to measure [^{14}C]-AP uptake by liquid scintillation counting. The result was expressed as CPM/mL of gland suspension. In all cases, protein was measured by Lowry *et al.* [24].

STATISTICAL EVALUATION. All the data were presented as mean ± SEM. Significance was calculated from Student's t-test.

RESULTS

MMI Activation of Parietal Cell

Measurement of O_2 consumption is one of the specific assays for H^+ secretion by activated parietal cells in the presence of any stimulant [19]. MMI stimulates O_2 consumption in a concentration-dependent manner showing a hyperbolic activation up to 200 μM (Fig. 1). At this optimum concentration O_2 consumption is increased by more than 2.5-fold ($P < 0.05$) over the basal value (9.2 ± 3 nmol/10^6 cell/min). PTU, another thionamide, also stimulates O_2 consumption similar to MMI, showing threefold stimulation ($P < 0.02$) at 200 μM. The rate of O_2 consumption by MMI(A) and PTU(B) is also shown in Fig. 1 inset. The potency of the thionamides to activate the parietal cell is compared with that of the known stimulants, e.g. histamine, IBMX and carbachol (Fig. 2). Although the potency of both the thionamides is comparable to that of histamine, they are more potent than IBMX ($P < 0.02$) or carbachol ($P < 0.05$). To study whether MMI stimulates

TABLE 1. Effect of antisecretagogues on MMI-stimulated O_2 consumption by isolated parietal cells

	Oxygen consumption (nmol/10^6 cells/min)
Control	9.2 ± 0.3
+ MMI	20 ± 1.6*
+ MMI + Cimetidine	18.2 ± 1.3†
+ MMI + Atropine	18.5 ± 2†
+ MMI + Propanolol	20.0 ± 3†
+ MMI + Omeprazole	10.2 ± 2*
+ MMI + Nifedipine	20.1 ± 2†
Omeprazole pretreated washed cells + MMI	11.2 ± 0.8*

Parietal cell (10^6) was incubated at 25°C in absence or presence of each drug (0.1 mM) in the oxygraph vessel for 20 min in 2 mL HBSS before the addition of 0.1 mM MMI. In a separate experiment, omeprazole-pretreated cell was washed off omeprazole and then used for MMI activation. Nifedipine was dissolved in absolute alcohol and protected from light (n = 4).

* $P < 0.01$ MMI vs. control MMI versus MMI + omeprazole and MMI vs. omeprazole pretreated washed cell + MMI.

† P not significant MMI vs. MMI + other drugs.

O_2 consumption by activating the receptors of the parietal cells, experiments were performed in presence of some receptor antagonists such as cimetidine (H_2-receptor blocker), atropine (muscarinic cholinergic receptor blocker) and propanolol (β-adrenergic receptor blocker). Table 1 shows that MMI stimulates O_2 consumption in the isolated parietal cell pretreated with cimetidine, atropine, propanolol, or nifedipine while it is inactive in omeprazole-pretreated cell. MMI also fails to increase O_2 consumption in the omeprazole-pretreated washed cells where omeprazole binds with the H^+-K^+-ATPase covalently. Thus, the inability of MMI to activate the parietal cell in presence of omeprazole is not due to depletion of MMI by acid-activated omeprazole in the parietal cell canaliculi. These results indicate that MMI acts neither through the histamine-H_2, muscarinic cholinergic, and β-adrenergic receptor nor through the voltage-sensitive Ca^{2+} entry mechanism, but this enhanced O_2 consumption is due to increased H^+ transport by the proton-pumping H^+-K^+-ATPase.

Parietal Cell Activation and Peroxidase–Catalase Inhibition by MMI and H_2O_2

As MMI is an established inhibitor of gastric peroxidase [16, 17], we looked for any correlation between acid secretion and peroxidase inactivation by MMI. Both MMI (Fig. 3A) and PTU-dependent activation of parietal cell (Fig. 3B) correlate well with the inhibition of the endogenous peroxidase activity, which is more than 90% with the 100 μM MMI or 200 μM PTU. It is expected that inhibition of peroxidase activity may lead to intracellular accumulation of H_2O_2, which might activate the parietal cell for increased acid secretion. Because catalase is also involved in the catabolism of endogenous H_2O_2, the parietal cell catalase activity was also investigated. Figure

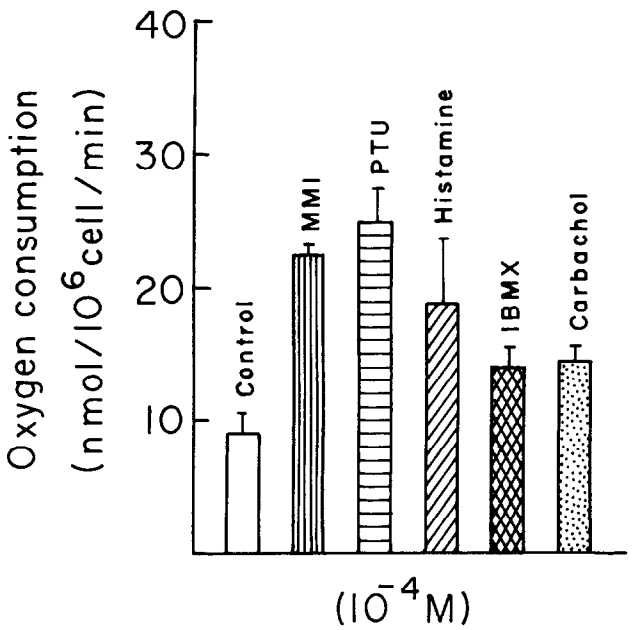

FIG. 2. Effect of parietal cell stimulants on O_2 consumption by isolated parietal cell. Detailed procedure was described in the text except that 0.1 mM of each of the test material was used. $n = 3$ for control group, 4 for thionamides and IBMX, and 6 for carbachol group. $P < 0.02$ for thionamides vs. IBMX, $P < 0.05$ for thionamides vs. carbachol, and P is not significant for thionamides vs. histamine.

4A shows the oxygraphic record for O_2 evolution by catalase-catalyzed decomposition of H_2O_2 by parietal cell homogenate. A slow rate of O_2 evolution dependent on the concentration of the homogenate and sensitive to azide was evident. In contrast, liver homogenate showed very high catalase activity (Fig. 4B). When the specific activity was calculated, parietal cell was found to contain very low catalase activity (0.17 μmol of O_2 evolved/min/mg) compared to liver (130 μmol/min/mg). As liver contains insignificant peroxidase activity (data not shown), it appears that intracellular H_2O_2 is scavenged mainly by catalase in liver, whereas it is utilized by the peroxidase in stomach. It is interesting to note that although the parietal cell catalase activity is low, it is inhibited with MMI when preincubated in presence of H_2O_2 (Table 2). MMI, at a concentration of 600 μM, inactivated the catalase activity by 82%. However, preincubation with MMI or H_2O_2 alone has no significant effect, indicating that catalytically active catalase is susceptible to inactivation by MMI. Table 2 further shows that the parietal cell peroxidase is also irreversibly inactivated [17] by MMI in the presence of H_2O_2, showing 90% inhibition at 200 μM MMI. Only MMI or H_2O_2 had no significant effect.

Effect of H_2O_2 on Acid Secretion

As inactivation of both peroxidase and catalase by MMI should elevate the intracellular concentration of H_2O_2, which may stimulate the acid secretion, we investigated the possible effect of H_2O_2 on acid secretion in isolated gastric

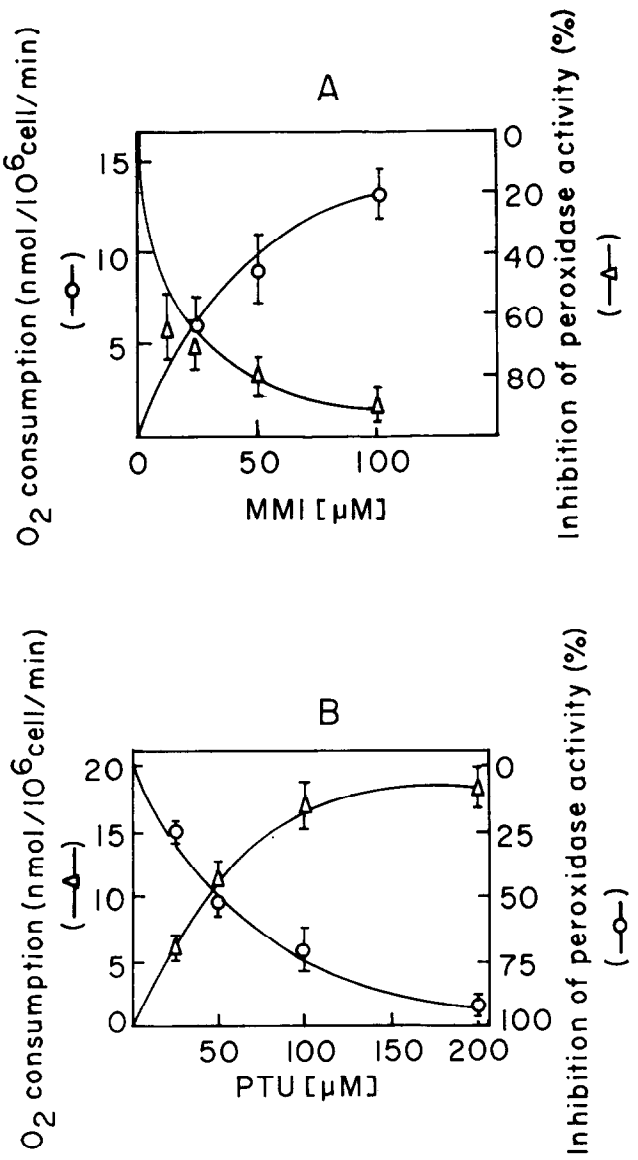

FIG. 3. Effect of MMI or PTU *in vitro* on O_2 consumption and peroxidase activity as measured by radioiodide organification in isolated parietal cell. The correlation of O_2 consumption and peroxidase activity was done against varying concentration of MMI or PTU 15 min after addition. The values for O_2 consumption were plotted after correction for basal value. (A) MMI (B) PTU ($n = 3$).

mucosa *in vitro*. Figure 5 shows that 5 μM H_2O_2 stimulates acid secretion by about sevenfold over the basal value. The stimulation persists at least for 20 min. Subsequent additions of H_2O_2 caused small but some increase in acid secretion until 60 μM is reached after which acid secretion started declining, presumably due to damage of the secretory machinery by higher H_2O_2 concentration. This increased acid secretion is also sensitive to omeprazole (Fig. 6). Addition of omeprazole after 20 min of H_2O_2 addition inhibits the stimulated acid secretion (A). However, in omeprazole-pretreated gastric mucosa, H_2O_2 cannot stimulate acid secretion significantly (B). Table 3 shows the effect of varying concentrations of MMI and H_2O_2 on

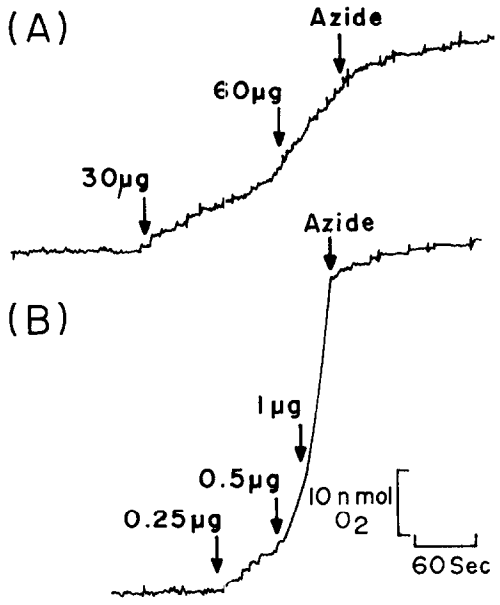

FIG. 4. Catalase activity of parietal cell and liver as measured by O_2 evolution. The indicated amount of the cell-free homogenate of liver or parietal cell was added to the oxygraph flask containing 2 mL of 50 mM phosphate buffer pH 7.2 and 100 μM H_2O_2. O_2 evolution was monitored immediately. Sodium azide (1 mM) was added to show catalase sensitivity. (A) Parietal cell; (B) liver. This is the result from a typical experiment and has been verified by two more experiments.

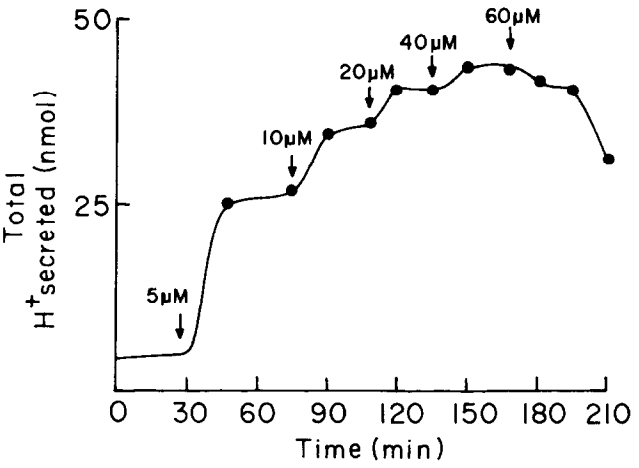

FIG. 5. Dose-dependent study of H_2O_2 on gastric acid secretion in Ussing chamber experiment. H^+ secretion was measured in the isolated gastric mucosa after addition of varying concentration of H_2O_2 to the serosal solution as indicated by arrows. This is the result of a typical experiment and has been verified by two more experiments.

[^{14}C]-AP accumulation in gastric gland preparation. MMI as low as 50 μM can stimulate the acid secretion by twofold, and the stimulation persists up to 200 μM. However, at 500 μM, the stimulation is decreased to some extent. H_2O_2, on the other hand, at a very low concentration of 1 μM, can stimulate acid secretion by more than threefold, but the stimulation decreased when the concentration is increased to 10 μM, possibly due to some damaging action of H_2O_2. When compared, histamine at an optimum concentration of 100 μM, is more effective stimulant for acid secretion in the isolated gastric gland preparation.

DISCUSSION

Many secretory compounds interact with the receptors at the basolateral membrane of the parietal cell leading to an increased level of second messengers such as cAMP, IP_3, and/or Ca^{2+}, which activates protein kinase and causes active transport of H^+ through the proton pumping H^+-K^+-ATPase [2–8]. However, the precise intracellular mechanism leading to the activation of H^+-K^+-ATPase is not clear yet. Although [^{14}C]-aminopyrine uptake is a well-established assay for acid secretion by parietal cell [25, 26], there is also well-documented positive correlation between O_2 consumption of the activated parietal cell and

TABLE 2. Effect of MMI on catalase and peroxidase activity of parietal cell

	Catalase activity (O_2 evolved nmols/min/mg protein)	Peroxidase activity Units/mg protein
Control	170 ± 10	70 ± 8
+ MMI (50 μM)	150 ± 7†	36 ± 5*
+ MMI (100 μM)	120 ± 10*	15 ± 3***
+ MMI (200 μM)	100 ± 8**	7 ± 2***
+ MMI (400 μM)	60 ± 3**	
+ MMI (600 μM)	30 ± 3***	

An aliquot of cell free homogenate was preincubated for 5 min with 100 μM H_2O_2 in presence of varying concentration of MMI at 25°C in a final volume of 100 μL containing 50 mM sodium phosphate buffer, pH 7.2. Finally it was transferred to the oxygraph flask containing 2 mL of 50 mM phosphate buffer, pH 7.2 and the reaction was started by adding 100 μM H_2O_2 to assay the catalase activity. In case of inactivation of peroxidase, incubation was carried out in 50 mM Tris-HCl buffer pH 8.0 for 3 min before assaying an aliquot for peroxidase activity by I_3^- assay ($n = 4$).

* $P < 0.05$ MMI vs. control.

** $P < 0.01$ MMI vs. control.

*** $P < 0.001$ MMI vs. control.

† P not significant MMI vs. control.

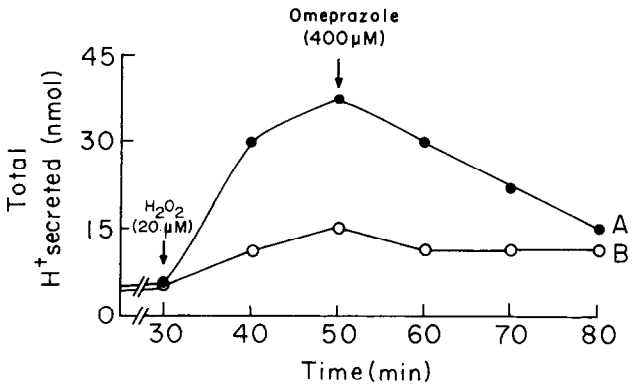

FIG. 6. Effect of omeprazole on H_2O_2-stimulated H^+ secretion in Ussing chamber experiment: 20 μM H_2O_2 was added to control and omeprazole (400 μM)-pretreated gastric mucosa and acid secretion was measured as described under Materials and Methods. (A) Control; (B) omeprazole pretreated. This is the result of a typical experiment and has been verified by two more experiments.

its H^+ secretion [19]. We have shown that MMI stimulates O_2 consumption, which correlates well with the inhibition of the peroxidase activity of the isolated parietal cell. Because MMI-induced O_2 consumption is insensitive to cimetidine, atropine, and propanolol, it is clear that MMI is not directly working through these receptors to modulate the signal transduction system for increased acid secretion. This is in contrast to our previous finding that MMI can stimulate acid secretion *in vivo*, which is sensitive to cimetidine [14]. This cimetidine-sensitive acid secretion may be due to increased histamine release from the adjacent mast cell either directly by MMI or indirectly through degranulating action on mast cell by H_2O_2 likely to be generated by peroxidase inhibition [27]. It is further shown that nifedipine, a calcium channel blocker, is unable to block the MMI-induced O_2 consumption, thus eliminating the involvement of Ca^{2+} entry through this channel as required in different receptor-mediated secretagogues-stimulated O_2 consumption [4]. However, this increased O_2 consumption by activation of parietal cell by MMI is coupled with the increased activity of the proton pump to actively secrete HCl, as evidenced by the sensitivity to omeprazole, the blocker of proton pumping H^+-K^+-ATPase [28, 29].

TABLE 3. Uptake of [^{14}C]-aminopyrine in isolated gastric gland by mercaptomethylimidazole and H_2O_2

	[^{14}C]-Aminopyrine uptake CPM
Control	450 ± 42
+ MMI (50 μM)	926 ± 78**
+ MMI (100 μM)	1006 ± 85**
+ MMI (200 μM)	1089 ± 100**
+ MMI (500 μM)	781 ± 80*
+ H_2O_2 (0.5 μM)	1240 ± 20***
+ H_2O_2 (1 μM)	1446 ± 40***
+ H_2O_2 (10 μM)	800 ± 38***
+ Histamine (100 μM)	1762 ± 50***

Gastric gland was prepared from rabbit gastric mucosa and was incubated for 30 min at 25°C with indicated concentration of MMI or H_2O_2 or histamine in presence of 1 μCi of [^{14}C]-aminopyrine. The uptake was measured as described under Materials and Methods (n = 4).

* $P < 0.02$ MMI vs. control.

** $P < 0.02$ MMI vs. control.

*** $P < 0.001$ H_2O_2 or histamine vs. control.

PTU, another thionamide, also activates the parietal cell similar to MMI through the inhibition of the peroxidase activity. We have studied the mechanism of inactivation of gastric peroxidase by MMI and found that it irreversibly inactivates the enzymes by acting as a suicidal substrate [17]. Further studies on lactoperoxidase indicated that MMI-thiyl radical, a one-electron oxidation product, interacts at the heme to cause irreversible inactivation [13]. It is expected that inhibition of peroxidase activity may lead to intracellular accumulation of H_2O_2, which might stimulate H^+ secretion.

Because catalase is also involved in scavenging the intracellular H_2O_2, the parietal cell catalase level was also checked. Although parietal cell contains low catalase activity compared to liver, it is also inhibited by MMI similar to gastric peroxidase [17]. Thus, MMI by inactivating both peroxidase and catalase, creates a favorable condition for the accumulation of endogenous H_2O_2.

Evidence is also presented to show that H_2O_2 can stimulate acid secretion in isolated gastric mucosa as well as in gastric gland preparations. The possibility exists that H_2O_2 when permeates into the cell, it may be scavenged by the active peroxidase–catalase system inside the parietal cell. It, thus, appears that H_2O_2, while diffusing through the cell membrane, may modulate the membrane signal transduction system at the cytosolic side before it is scavenged by these enzymes. In MMI-treated cells, when these enzymes are inactivated, the increased intracellular H_2O_2 may diffuse from the cytosol to affect the signal transduction system [27]. The alternate possibility may also exist that increased H_2O_2 level generates hydroxyl radical in presence of O_2^- and the transition metal ions through the metal-catalysed Haber-Weiss reaction [30]. Hydroxyl radical being highly reactive, may interact with the regulatory system to activate acid secretion. However, the presence of free metal ion is a prerequisite for this effect. As very high concentration of H_2O_2 is toxic to the cell, parietal cell peroxidase may normally utilize this oxygen metabolite to perform some specialized function. We have recently shown that gastric peroxidase can effectively scavenge the endogenous H_2O_2 using SCN^- as a major electron donor in concert with GSH, NADPH, and glutathione reductase [31]. Evidence is accumulating on the role of increased cellular H_2O_2 in the alteration of various cellular functions also [32]. Several investigators have suggested the role of H_2O_2 in the cellular response to insulin [33–35]. Cross *et al.* [36] have proposed that H_2O_2 is an intermediary in the transduction of arterial O_2 pressure (pO_2) into modulation of neuronal activity in chemosensitive neurons in the carotid body. H_2O_2 may also modulate the K^+ channel of neuronal cells, thereby regulating excitability and affecting

K^+ current in response to changes in pO_2 [37]. Very recently, intracellular H_2O_2 has been reported to activate agonist-sensitive Ca^{2+} flux in canine venous endothelial cell [38], where H_2O_2 has been suggested to act as an intracellular messenger. We propose that accumulation of intracellular H_2O_2 due to inactivation of the peroxidase–catalase system of the parietal cell by MMI, before reaching the critical damaging level, may act like a second messenger or may modulate the signal transduction system of the parietal cell for induction of acid secretion.

Recently, H_2O_2 has been shown to inhibit the prostaglandin synthetase activity of the aortic endothelial cell [39]. It is possible that elevated intracellular H_2O_2 may stimulate acid secretion by decreasing the biosynthesis of prostaglandin, the natural inhibitor of acid secretion [40], through inactivation of the prostaglandin synthetase [41]. Further studies are required to prove this. Recent studies have opened up a new role of moderate concentration of intracellular H_2O_2 and cellular redox state on gene expression and posttranslational modification of proteins [42] as well as on the perturbation of intracellular Ca^{2+} homeostasis [43]. H_2O_2 may act as a signal transduction messenger or affect the critical steps in the signal transduction cascade, thereby affecting the basic events of cellular regulation [44]. It is not unlikely that mercaptomethylimidazole or similar drug-induced hyperacidity is caused by change in the redox (oxidant–antioxidant homeostasis) status of the parietal cell. Although it is tempting to speculate the possible role of the elevated H_2O_2 on acid secretion transduction pathway of the parietal cell, this hypothesis will be substantiated after direct demonstration of the increased intracellular level of H_2O_2 by MMI as well as after identification of any component of the signal transduction system sensitive to H_2O_2. Works are in progress in these directions.

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References

1. Forte JG and Machen TE, Ion transport by gastric mucosa. In: *Physiology of Membrane Disorders* (Eds. Andreoli TE, Hoffman JF, Fanestil DD and Schultz SG) pp. 535–558. Plenum, New York, 1986.
2. Soll AH and Wollin A, Histamine and cyclic AMP in isolated canine parietal cells. *Am J Physiol* **237:** E444–E450, 1979.
3. Berglindh T, Sachs G and Takeguchi N, Ca^{2+}-dependent secretagogue stimulation in isolated rabbit gastric glands. *Am J Physiol* **239:** G90–G94, 1980.
4. Chew CS and Brown MR, Release of intracellular Ca^{2+} and elevation of inositoltriphosphate by secretagogues in parietal and chief cells isolated from rabbit gastric mucosa. *Biochim Biophys Acta* **888:** 116–125, 1986.
5. Shlatz LJ, Bools C and Reiman EM, Phosphorylation of membrane from the rat gastric mucosa. *Biochim Biophys Acta* **673:** 539–545, 1981.
6. Chew CS, Parietal cell protein kinase. *J Biol Chem* **260:** 7540–7550, 1985.
7. Sachs G, Chang HH, Rabon E, Schackmann R, Lewin M and Saccomani G, A non-electrogenic H^+ pump in plasma membrane of hog stomach. *J Biol Chem* **251:** 7690–7698, 1976.
8. Forte JG, Machen TE and Obrink KJ, Mechanism of gastric H^+ and Cl^- transport. *Annu Rev Physiol* **42:** 111–126, 1980.
9. Green WL, Mechanism of action of antithyroid compounds. In: *The Thyroid*, 3rd ed. (Eds. Werner SC and Ingbar SH) pp. 41–49. Harper and Row, New York, 1971.
10. Engler H, Taurog A and Nakashima T, Mechanism of inactivation of thyroid peroxidase by thioureylene drugs. *Biochem Pharmacol* **31:** 3801–3806, 1982.
11. Nakamura S, Nakamura M, Yamazaki I and Morrison M, Reactions of ferryl lactoperoxidase (compound II) with sulfide and sulfhydryl compounds. *J Biol Chem* **259:** 7080–7085, 1984.
12. Doerge DR, Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogen. *Biochemistry* **25:** 4724–4728, 1986.
13. Bandyopadhyay U, Bhattacharyya D, Chatterjee R and Banerjee RK, Irreversible inactivation of lactoperoxidase by mercaptomethylimidazole through generation of a thiyl radical: Its use as a probe to study the active site. *Biochem J* **306:** 751–757, 1995.
14. Bhattacharjee M, Bose AK and Banerjee RK, Histamine H_2-receptor mediated stimulation of gastric acid secretion by mercaptomethylimidazole. *Biochem Pharmacol* **38:** 907–914, 1989.
15. De SK and Banerjee RK, Purification, characterization and origin of rat gastric peroxidase. *Eur J Biochem* **160:** 319–325, 1986.
16. Bandyopadhyay U, Bhattacharyya DK, Chatterjee R and Banerjee RK, Localization of gastric peroxidase and its inhibition by mercaptomethylimidazole, an inducer of gastric acid secretion. *Biochem J* **284:** 305–312, 1992.
17. Bandyopadhyay U, Bhattacharyya DK and Banerjee RK, Mechanism-based inactivation of gastric peroxidase by mercaptomethylimidazole. *Biochem J* **296:** 79–84, 1993.
18. Thompson WJ, Chang LK and Rosenfeld GC, Histamine regulation of adenyl cyclase of enriched rat gastric parietal cells. *Am J Physiol* **240:** G76–G84, 1981.
19. Berglindh T, Effect of common inhibitors of gastric acid secretion on secretagogue-induced respiration and aminopyrine accumulation in isolated gastric glands. *Biochim Biophys Acta* **464:** 217–233, 1977.
20. Goldstein DB, A method for assay of catalase with the oxygen cathode. *Anal Biochem* **24:** 431–437, 1968.
21. Reeves JJ and Stables R, Effect of indomethacin, piroxicam and selected prostanoids on gastric acid secretion by the rat isolated gastric mucosa. *Br J Pharmacol* **86:** 677–684, 1985.
22. Berglindh T and Obrink KJ, A method for preparing isolated glands from the rabbit gastric mucosa. *Acta Physiol Scand* **96:** 150–159, 1976.
23. Berglindh T, Helander HF and Obrink KJ, Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta Physiol Scand* **97:** 401–414, 1976.
24. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
25. Berglindh T, Dibona DR, Ito S and Sachs G, Probes of parietal cell function. *Am J Physiol* **238:** G165–G176, 1980.
26. Soll AH, Specific inhibition by prostaglandins E_2 and I_2 of histamine-stimulated [^{14}C]-aminopyrine accumulation and cyclic adenosine monophosphate generation by isolated canine parietal cells. *J Clin Invest* **65:** 1222–1229, 1980.
27. Banerjee RK, Das PK and Bhattacharjee M, Gastric peroxidase and its role in cellular control of gastric acid secretion. In: *Biological Oxidation Systems* (Eds. Reddy CC, Hamilton

GA and Madyastha KM), Vol. 1 pp. 505–513. Academic Press Inc., San Diego, 1990.
28. Fellenius I, Berglindh T, Sachs G, Olbe L, Elander B, Sjostrand SE and Wallmark B, Substituted benzimidazoles inhibit gastric acid secretion by blocking ($H^+ + K^+$) ATPase. *Nature* **290:** 159–161, 1981.
29. Wallmark B, Larsson H and Humble L, The relation between gastric acid secretion and gastric H^+ K^+-ATPase activity. *J Biol Chem* **260:** 13681–13684, 1985.
30. Halliwell B and Gutheridge JMC, Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol* **186:** 1–85, 1990.
31. Das D, De PK and Banerjee RK, Thiocyanate—A plausible physiological electron donor of gastric peroxidase. *Biochem J* **305:** 59–64, 1995.
32. Ramasarma T, Generation of H_2O_2 in biomembranes. *Biochim Biophys Acta* **694:** 69–93, 1982.
33. Czech MP, Lawrence JC Jr and Lynn WS, Evidence for the involvement of sulfhydryl oxidation in the regulation of fat cell hexose transport by insulin. *Proc Natl Acad Sci USA* **71:** 4173–4177, 1974.
34. Czech MP, Lawrence JC Jr and Lynn WS, Hexose transport in isolated brown fat cells. A model system for investigating insulin action on membrane transport. *J Biol Chem* **253:** 2104–2113, 1978.
35. Muchmore DB, Little SA and Haen C, Counterregulatory control of intracellular hydrogen peroxide production by insulin and lipolytic hormones in isolated rat epididymal fat cells: A role of free fatty acid. *Biochemistry* **21:** 3886–3892, 1982.
36. Cross AR, Henderson LTG, Jones O, Delpiano MA, Hentschel J and Acker H, Involvement of an NADPH oxidase as a pO_2 sensor protein in the rat carotid body. *Biochem J* **272:** 743–747, 1990.
37. Vega-Saenz de Miera E and Rudy B, Modulation of K^+ channels by hydrogen peroxide. *Biochem Biophys Res Commun* **186:** 1681–1687, 1992.
38. Doan NT, Gentry DL, Taylor AA and Elliott SJ, Hydrogen peroxide activates agonist-sensitive Ca^{2+} flux pathways in canine venous endothelial cells. *Biochem J* **297:** 209–215, 1994.
39. Buckley BJ, Kent RS and Whorton RA, Regulation of endothelial cell prostaglandin synthesis by glutathione. *J Biol Chem* **266:** 16659–16666, 1991.
40. Rosenfeld CG, Prostaglandin E_2 inhibition of secretagogue-stimulated [^{14}C]-aminopyrine accumulation in rat parietal cells: A model for its mechanism of actions. *J Pharmacol Exp Ther* **237:** 513–518, 1986.
41. O'Brien PJ and Rahimtula A, The possible involvement of a peroxidase in prostaglandin biosynthesis. *Biochem Biophys Res Commun* **70:** 832–838, 1976.
42. Sies H, Oxidative stress. In: *Oxidants and antioxidants*. pp. 650. Academic Press, London, 1991.
43. Trump BF and Berezesky IK, The role of cytosolic Ca^{2+} in cell injury, necrosis and apoptosis. *Curr Opin Cell Biol* **4:** 227–232, 1992.
44. Sen CK and Packer L, Antioxidant and redox regulation of gene transcription. *FASEB J* **10:** 709–720, 1996.