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Inhibition of Gastric Mucosal Prostaglandin Synthetase Activity by Mercaptomethylimidazole, an Inducer of Gastric Acid Secretion—Plausible Involvement of Endogenous H_2O_2

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ABSTRACT. We have reported earlier that mercaptomethylimidazole (MMI), an antithyroid drug of thionamide group, induces gastric acid secretion at least partially through the liberation of histamine, sensitive to cimetidine. Now, we show that the drug has a significant inhibitory effect on the cyclooxygenase and peroxidase activity of the prostaglandin (PG) synthetase of the gastric mucosal microsomal preparation. The effect can also be mimicked by low concentrations of H_2O_2. While studying the possible intracellular effect of MMI on acid secretion, a cell fraction (F_3) enriched in parietal cell was isolated by controlled digestion of the mucosa with protease. This cell fraction is activated by MMI as measured by increased O_2 consumption. The activation is sensitive to omeprazole, a proton-pump inhibitor, indicating that the activation is due to increased acid secretion by MMI. MMI was also found to directly inhibit the peroxidase activity of the F_3 cell fraction and may thus increase the intracellular level of H_2O_2. The cyclooxygenase activity of the PG synthetase of the F_3 cell fraction is also inhibited by MMI and the effect can be reproduced by low concentrations of H_2O_2. Both MMI and H_2O_2 can also inhibit the peroxidase activity of the PG synthetase. We suggest that in addition to the activation of the parietal cell by MMI possibly through endogenous H_2O_2, MMI induces acid secretion *in vivo* by inactivating the PG synthetase thereby inhibiting the biosynthesis of PG and removing its inhibitory influence on acid secretion so that the histamine released by MMI can stimulate acid secretion with maximum efficiency. BIOCHEM PHARMACOL **56**;7:905–913, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. mercaptomethylimidazole and gastric acid secretion; gastric prostaglandin synthetase; gastric peroxidase, parietal cell

Gastric acid (HCl) secretion is stimulated by several physiological secretagogues such as histamine, acetylcholine and gastrin which act through the specific receptor on the parietal cell [1]. The stimulus-secretion coupling takes place through the involvement of a second messenger such as cAMP, Ca^{2+} or inositol trisphosphate which activates various cellular protein kinases [2]. The message is transmitted to the terminal proton pumping H^+-K^+-ATPase at the apical membrane which actively transports H^+ in exchange with K^+ [3, 4]. The molecular mechanism of the stimulus-secretion coupling has not been fully resolved yet. However, various cellular and cytoskeletal proteins might play an important role in the regulation of gastric acid secretion [5–7].

Among various effectors, PG† is known to play an important role in the regulation of gastric acid secretion. PG E_2 inhibits histamine stimulated acid secretion via the inhibitory GTP binding protein (G_i) of the adenylate cyclase [8–10]. Enzymes responsible for the biosynthesis and degradation of the PGs have been identified in the epithelial wall of the stomach. It is now well established that PG acts as a natural inhibitor of acid secretion and stimulator of cytoprotection against various damaging factors causing gastric ulceration [11]. PG is synthesised from arachidonic acid by PG endoperoxide synthase having two enzymatic activities [12]. The initial step is the oxygenation of arachidonic acid catalysed by the COX part of the PG synthetase yielding PGG_2 which is subsequently reduced to PGH_2 by the peroxidase part of the enzyme. Recently, *de novo* synthesis of the enzyme has been shown in guinea pig gastric mucous cells [13]. The enzyme exists in two forms—COXI and COXII. COXI is a constitutive enzyme while COXII is inducible and found only in a limited number of cells involved in inflammation and proliferation [14]. Both isomers have COX and peroxidase activity [15]. NSAIDs

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† *Abbreviations:* COX, cyclooxygenase; ESA, eicosatrienoic acid; GPO, gastric peroxidase; HBSS, Hank's balanced salt solution; MMI, mercaptomethylimidazole; NSAIDs, nonsteroidal anti-inflammatory drugs; and PG, prostaglandin.

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inhibit PG biosynthesis and stimulate acid secretion by inhibiting the activity of the PG synthetase [16]. Apart from the NSAIDs, we have shown that MMI, an antithyroid drug of thionamide group acts as a potent inducer of gastric acid secretion [17]. 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 the H^+-K^+-ATPase [17]. Although MMI stimulates histamine release which accounts for the cimetidine sensitivity [18], the partial inhibition by cimetidine also indicates possible involvement of some intracellular site of action of MMI. While studying the mechanism, MMI induced acid secretion was found to be correlated with the inhibition of the GPO [19, 20] which is located mainly in the parietal cell [20] and is involved in the scavenging of the endogenous H_2O_2 [21]. Further studies indicated that MMI inactivates the purified GPO by acting as a suicidal substrate [22] and may thus help in the accumulation of endogenous H_2O_2. We have also shown recently that MMI can directly inactivate the parietal cell peroxidase and catalase activity [23] and exogenous H_2O_2 at low concentration can induce acid secretion in isolated gastric mucosal preparation as well as in the gastric gland [23]. We suggested that MMI induced acid secretion is mediated through endogenous accumulation of H_2O_2 which probably acts like a second messenger to activate signal transduction mechanism [23]. As PG synthetase plays an important role in controlling acid secretion by generating PG, possibility exists that either MMI or H_2O_2 may directly inhibit this enzyme to stimulate acid secretion optimally by histamine released by MMI [18]. From the results presented in this communication, we suggest that MMI induced acid secretion by liberated histamine *in vivo* [18] occurs optimally by inactivation of the PG synthetase either directly or indirectly through increased intracellular level of H_2O_2 following peroxidase inhibition.

MATERIALS AND METHODS

Materials

Protease (type XIV), thiobarbituric acid, porcine pepsin, epinephrine, eicosatrienoic acid, BSA, histamine and MMI were purchased from Sigma. HBSS was prepared according to the instruction of the Life Technologies manual. Omeprazole was a gift from Dr. W. Beil of Medizinische Hochschule, Hannover, Germany. All other reagents used were of analytical grade.

Methods

PREPARATION OF MITOCHONDRIAL FRACTION FROM MOUSE STOMACH FOR THE ASSAY OF PEROXIDASE ACTIVITY. Fundic stomachs from Balb/c mice (20–25 g) of Institute inbred strain were minced and a 5% homogenate was prepared by homogenising in a motor-driven Potter–Elvehjem glass homogeniser for 60 sec in a solution containing 0.25 M sucrose and 50 mM potassium phosphate buffer pH 7.2. The homogenate was centrifuged at 1000 *g* for 10 min to remove cell debris and nuclear fraction and the supernatant was recentrifuged at 10,000 *g* for 10 min in a Sorvall RC5B refrigerated centrifuge to obtain the mitochondrial fraction. The pellet was suspended in 1 mL of the sucrose-phosphate buffer and peroxidase activity was assayed by following the formation of I_3^- at 353 nm using iodide as an electron donor [19]. The assay system contained in a final volume of 1 mL; 50 mM sodium acetate buffer pH 5.2, 1.7 mM KI and a suitable amount of enzyme preparation. H_2O_2 (0.3 mM) was added last to start the reaction. Results were expressed either as total activity (ΔOD/min of total protein) or specific activity (ΔOD/min/mg of protein). This fraction has no significant PG synthetase COX activity as measured by O_2 consumption in presence of ESA (described below) indicating that mitochondrial peroxidase [19] is not contributed by the PG synthetase peroxidase.

PREPARATION OF MICROSOMAL FRACTION FROM MOUSE STOMACH FOR THE ASSAY OF PG SYNTHETASE ACTIVITY. Microsomal fraction was prepared from fundic stomach of 12 mice as described previously [24]. Briefly, fundic stomachs were minced and homogenised in 30 mL of 50 mM potassium phosphate buffer pH 7 for 60 sec. The homogenate was centrifuged at 10,000 *g* for 15 min. The supernatant was further centrifuged at 105,000 *g* for 60 min. The microsomal pellet was suspended in 1 mL of 10 mM potassium phosphate, buffer pH 7 and was used immediately for the assay of PG synthetase activity. PG synthetase has both COX and peroxidase activity [24–26]. The peroxidase activity of the enzyme was measured spectrophotometrically as described [26]. The reaction mixture contained in a final volume of 1 mL: 50 mM Tris-HCl buffer pH 8, 1 mM epinephrine and a suitable amount of the microsomal protein. Auto-oxidation of epinephrine was minimised by the inclusion of 1 mM EDTA. The reaction was started by the addition of H_2O_2 (0.5 mM) and the activity was measured by recording the increase in absorbance at 480 nm due to formation of adrenochrome. Results were expressed as nmol of adrenochrome formed/min/mg of protein. The COX activity was measured in a Gilson oxygraph by monitoring the oxygen consumption [25] following cyclooxygenation of ESA. The reaction mixture contained 0.2 M Tris-HCl buffer pH 8, 0.5 mM epinephrine and a suitable volume of the enzyme preparation. The reaction was started by adding 75 μg of ESA. The initial rate of O_2 consumption was expressed as nmol of O_2 consumed/min/mg of protein. The microsomal fraction has no significant peroxidase activity as measured by highly sensitive iodide oxidation assay [19] and hence the peroxidase activity as measured by epinephrine oxidation [26] is contributed by the PG synthetase.

PREPARATION OF DISPERSED CELL SUSPENSION FROM STOMACH. Dispersed cell suspension from mouse stomach was prepared after modification of previous method [27, 28]. The fundic stomachs of eight mice were rinsed with chilled oxygenated HBSS, pH 7.4, the mucosa were pooled,

minced well, washed with HBSS and transferred to a double-walled glass chamber (for water circulation at a constant temperature) containing 12 mL of HBSS ($NaHCO_3$-Ca^{2+}) with 0.1% BSA and 0.5 mg/mL of protease (type XIV). The tissue was digested by slow stirring with constant oxygenation. After 30 min, the tissue was allowed to settle. The supernatant was decanted through nylon mesh and centrifuged at 4,000 *g* for 1 min. The resulting cell pellet was washed with oxygenated HBSS twice to remove adhering protease and termed as F_1 fraction. The remaining tissue was resuspended in 12 mL of HBSS with 0.1% BSA and 0.5 mg/mL of protease and digested for a further 45 min. The whole process was repeated as above to give a cell suspension designated as F_2. The remaining tissue was similarly processed for another 2 hr. The resulting cell fraction was termed as F_3 fraction. Each cell fraction was suspended in oxygenated HBSS and kept at room temperature for 30 min for further studies. The viability of the cell was checked by dye exclusion using trypan blue. Both viability and cell count were done in a phase contrast microscope with a magnification of ×400.

O_2 CONSUMPTION OF THE ISOLATED CELL. Activation of cell was measured by O_2 consumption according to the method of Berglindh [29]. After 30 min of incubation at 37°, 10^6 cells were added in a final volume of 2 mL of HBSS with BSA (0.1%) and calcium (1 mM) to the flask of the Gilson oxygraph and O_2 consumption was measured in presence and absence of the desired agent as described in the legend of each table and figure. Consumption of O_2 was expressed as nmol of O_2 consumed per min per 10^6 cells.

ESTIMATION OF CELLULAR MUCIN. The cell fractions were suspended in 0.1 N H_2SO_4 and sonicated at low energy (two pulses of 10-sec duration). The fractions were heated at 80° for 1 hr to release the bound sialic acid which was estimated with thiobarbituric acid assay method [30]. Briefly, to 0.2 mL of the sample was added 0.1 mL of 0.2 mM periodate solution and shaken well. This was allowed to stand at room temperature for 20 min followed by the addition of 1 mL of 10% arsenite solution. It was shaken until the yellow brown colour disappeared. To this was added 3 mL of 0.6% thiobarbituric acid, shaken and heated in a boiling water bath for 15 min. To it, 4.3 mL of cyclohexanone was added, shaken well and centrifuged for 3 min at 1,000 *g*. The optical density of the clear upper phase was read at 549 nm. The amount of sialic acid was determined from a standard curve and expressed as total mucin content per mg of cell protein.

CELLULAR PEPSIN ACTIVITY. Cells of each fraction were homogenised in 50 mM potassium phosphate buffer pH 7.4 after freeze-thawing twice. The homogenate was centrifuged at 15,000 *g* for 10 min and the supernatant was used for the pepsin assay [31]. An aliquot (0.1 mL) was incubated with 0.2 N HCl and 0.06 M KCl in presence of 1 mL of 2.5% BSA in a total volume of 2 mL for 30 min at 37°.

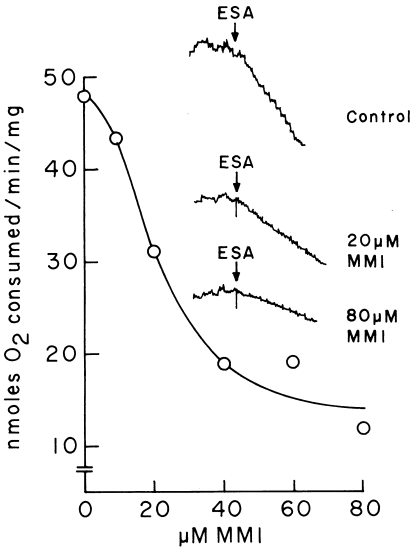

FIG. 1. Effect of varying concentrations of MMI on the PG synthetase (COX) activity of the microsomal fraction of mouse stomach. COX activity was calculated from the initial rate of O_2 consumption in absence and presence of varying concentrations of MMI. Inset shows the typical oxygraphic tracings in absence or presence of MMI. The result is from a representative experiment and has been verified by two more experiments.

The protein was precipitated with 2 mL of 0.3 M TCA and kept in ice bath for 30 min. The precipitate was removed by filtration through Whatman 3 MM paper. The clear filtrate containing small peptides was assayed at 280 nm as a measure of pepsin activity. The result was expressed as units of pepsin activity, using porcine pepsin as a standard. The specific activity was expressed as units/mg of protein. Protein was estimated according to the method of Lowry *et al.* [32].

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

RESULTS

Effect of MMI on Gastric Mucosal PG Synthetase Activity

As PG acts as a natural inhibitor of acid secretion, the effect of MMI was studied on the PG synthetase activity of

TABLE 1. Comparison of the effect of MMI with indomethacin—a COX inhibitor of the PG synthetase

	PG synthetase COX activity nmol O_2 consumed/min/mg
Control	38 ± 5.0
+ MMI 100 μM	10 ± 1.5
+ Indomethacin 100 μM	8 ± 1.0

The PG synthetase COX activity of the gastric mucosal microsomal fraction was determined by O_2 consumption in absence or presence of MMI or indomethacin as described in the text. The enzyme was incubated with the indicated concentration of the reagent for 5 min in the oxygraph vessel before the addition of eicosatrienoic acid and epinephrine.

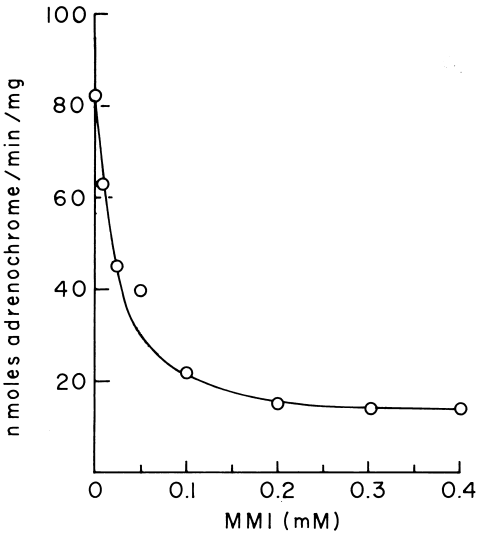

FIG. 2. Effect of varying concentrations of MMI on the PG synthetase-peroxidase activity of the microsomal fraction of mouse stomach. The activity was measured by the formation of adrenochrome at 480 nm in presence of varying concentrations of MMI as indicated. The result is from a representative experiment and has been verified by two more experiments.

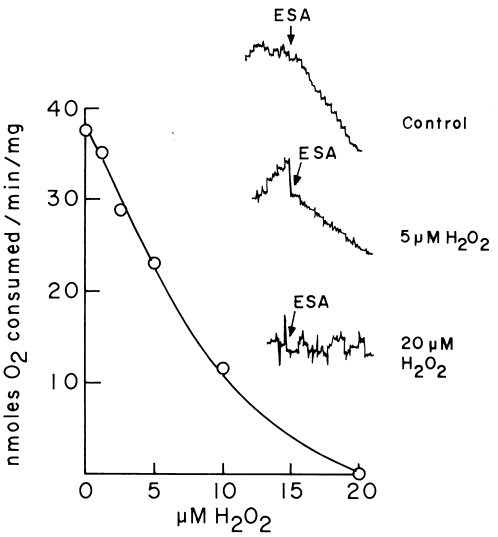

FIG. 3. Effect of varying concentrations of H_2O_2 on the PG synthetase-COX activity of the microsomal fraction of mouse stomach. The enzyme was preincubated with the indicated concentrations of H_2O_2 for 5 min before assaying the rate of O_2 consumption. The inset shows the results of the typical oxygraphic records in absence and presence of H_2O_2 and have been verified by two more experiments.

the gastric mucosal microsomal fraction (Fig. 1). The inset shows the oxygraphic tracing of O_2 consumption in presence of varying concentrations of MMI. MMI directly inhibits the COX activity in a concentration-dependent manner with IC_{50} of 30 μM and showing nearly 70% inhibition with 80 μM. When compared (Table 1), MMI was found to be almost as effective as indomethacin, an established inhibitor of the COX activity of the PG synthetase [26]. Figure 2 shows that peroxidase activity of the PG synthetase of the same microsomal fraction is also inhibited by MMI. Nearly 75% inhibition was observed with 0.1 mM MMI with IC_{50} of 50 μM. As MMI inactivates the peroxidase-catalase system of gastric mucosa [22, 23] and should increase the endogenous H_2O_2 level, the possible role of H_2O_2 on the PG-synthetase activity was therefore studied. The result (Fig. 3 and the inset) shows that COX activity of the PG synthetase gradually decreased with increasing concentration of H_2O_2 with IC_{50} of 6.3 μM and showing almost complete inhibition with 20 μM.

Effect of MMI on the Isolated Parietal Cell

In order to study the intracellular mechanism of action, MMI effect was studied on the parietal cell isolated from the gastric mucosa. During sequential enzymatic digestion of the fundic mucosa, three cell fractions (F_1, F_2 and F_3) were isolated (Table 2). Mucin secreting cell, chief cell and parietal cell were judged by mucin content, pepsin activity and histamine-stimulated O_2 consumption, respectively. As revealed in Table 2, F_1, F_2 and F_3 fractions are functionally distinct. F_1 had a yield of 2×10^6 cells/stomach and was enriched with the surface epithelial cell secreting mucin. F_2 had an yield of 6×10^5 cells/stomach and contained a mixture of chief cell, a few surface epithelial cell and some parietal cell. These fractions did not show significant histamine-stimulated O_2 consumption. F_3 had an yield of 1.4×10^6 cells/stomach of which the majority were large (15 μ) and readily identifiable as parietal cell as judged by established morphological criteria by their comparatively

TABLE 2. Differentiation of the three fractions of dispersed cell suspension from mouse gastric mucosa by biochemical indices

Cell fraction	Mucin Content		Pepsin		Consumption/ min/10^6 cells	O_2
	Total (μg)	μg/ mg**	Total activity (units)	Specific activity unit/mg**	−Hist	+Hist 10^{-4} M
F_1	7.6	6.4	5.5	3.2	1.65 ± 0.4	1.85 ± 0.3
F_2	1.8	3.7	4.2	8.2	1.56 ± 0.7	2.00 ± 0.7
F_3	5.3	3.4	13	8.1	2.15 ± 0.86	3.8 ± 1.2*

The cells from the fractions were processed and measured for either mucin content or pepsin activity as described in Materials and Methods. For O_2 consumption studies, cells were added to the incubation cell of the oxygraph, containing HBSS (pH 7.4) and 1 mM Ca^{2+} as described under Materials and Methods. The data of mucin and pepsinogen content are the mean of two experiments. O_2 consumption study is the mean of 4 separate experiments.

*$P < 0.05$; **mg of cell protein.

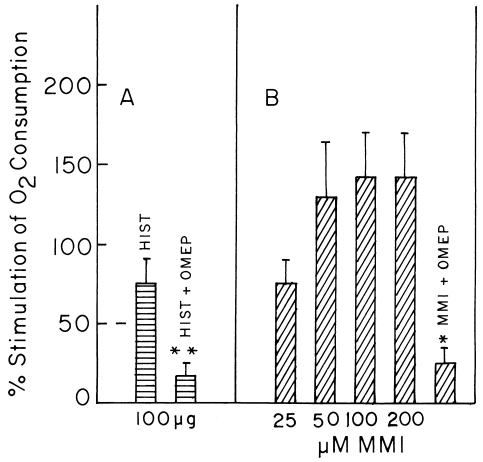

FIG. 4. Effect of histamine and MMI on oxygen consumption in F_3 cell fraction. F_3 cell fraction was obtained as described under Materials and Methods. Oxygen consumption was expressed as the mean percentage stimulation by histamine or MMI above the basal value ± SEM. The concentration of omeprazole used was 100 µM. *P < 0.01; **P < 0.02; N = 3.

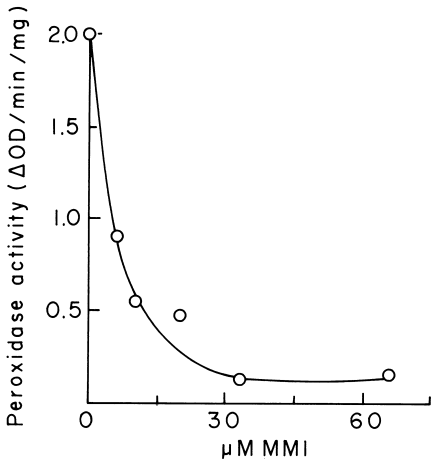

FIG. 5. Effect of varying concentrations of MMI on GPO activity of the mitochondrial fraction of the F_3 cells. Peroxidase activity was measured by following the formation of I_3^- at 353 nm in absence or presence of varying concentrations of MMI. The result presented is from a typical experiment and has been verified by two more experiments.

larger diameter, concentric nuclei and eosinophilic cytoplasm. Predominance of parietal cell was observed in F_3 fraction as shown by its significant stimulation of O_2 consumption by histamine. The purity of the parietal cell in this fraction is about 60%. It could not be purified further by density gradient centrifugation due to low yield. As parietal cell is activated during secretagogue-stimulated acid secretion as shown by increased O_2 consumption [29], the ability of the F_3 cell fraction to respond to histamine or MMI was first investigated. As shown in Fig. 4, F_3 cell fraction showed increased O_2 consumption when activated by histamine and O_2 consumption is significantly reduced by prior incubation with omeprazole (panel A). The cells, however, respond better than histamine when incubated with varying concentrations of MMI (panel B). This is due to acid secretion as revealed by its sensitivity to omeprazole. The result indicates that MMI can directly activate the parietal cell population of the F_3 fraction for acid secretion.

Effect of MMI and H_2O_2 on Peroxidase and PG Synthetase Activity of the F_3 Cell Fraction

MMI has been shown to be an established inhibitor of GPO [20, 22, 23]. The enzyme is highly enriched in F_3 cell fraction showing highest specific activity and having 65–75% of total activity (Table 3). Assay of the mitochondrial peroxidase activity of the F_3 cell fraction with MMI shows concentration dependent inhibition of the enzyme activity (Fig. 5) which is 95% inhibited by 32 µM MMI with an IC_{50} value of 7 µM. These three cell fractions also showed significant PG synthetase COX activity in the microsomal fraction as revealed by O_2 consumption (Table 4). F_2 cells contain the highest enzyme activity followed by F_3 and F_1 fractions. Figure 6 shows the effect of MMI on the COX activity of the F_3 cell fraction. The result indicates that MMI can directly inhibit the COX activity in a concentration-dependent manner showing an IC_{50} value of 20 µM whereas nearly 80% loss of activity occurs with 100 µM MMI. The inset show the oxygraphic tracing of O_2 consumption in absence and presence of MMI. As inactivation of peroxidase by MMI should increase intracellular level of H_2O_2 (catalase activity of this cell fraction is very low), the effect of latter was also studied on the microsomal PG synthetase activity of the F_3 cell fraction. As shown in Fig. 7, preincubation with H_2O_2 causes a concentration-dependent irreversible inactivation of the COX part of the enzyme which shows an IC_{50} value at 4 µM. The inset shows the rate of O_2 consumption in absence or presence of H_2O_2. Besides COX, the peroxidase activity of the microsomal PG synthetase of the F_3 cells is also inhibited by both MMI and H_2O_2 (Table 5). Mercaptomethylimidazole 100 µM or preincubation with 20 µM H_2O_2 can cause around

TABLE 3. GPO activity in the three fractions of dispersed cell suspension from mouse gastric mucosa

	Specific activity ΔOD/min/mg			Total activity		
Experiment	F_1	F_2	F_3	F_1	F_2	F_3
1	3.7	7.2	20	11.5	9	40
2	12	14	92.8	28	16	120
3	3	4	11.1	10.6	6	30

The cells of the three fractions F_1, F_2 and F_3 were homogenised after freeze-thawing. GPO activity of the homogenate was measured as described under Materials and Methods.

TABLE 4. PG synthetase (COX) activity in dispersed cells of mouse gastric microsomal fraction

	nmol O_2 consumed/min/mg	*N*
F_1	68.95 ± 12.7	4
F_2	288.5 ± 21.7	4
F_3	206.0 ± 25.7	4

PG synthetase (COX) activity of microsomal fraction of F_1, F_2 and F_3 fractions of cells was calculated from the initial rate of O_2 consumption as described in Materials and Methods.

95% inactivation of the peroxidase part of the PG synthetase. The H_2O_2 effect seems to be specific to the peroxidase part of the PG synthetase as preincubation of mitochondrial peroxidase with 20 μM H_2O_2 alone has no significant effect (data not shown). Moreover, GPO and PG synthetase peroxidase are two entirely different entity as evidenced by differential sensitivity to MMI (Table 6), GPO being more sensitive than PG synthetase peroxidase. Moreover, H_2O_2-preincubated microsomal fraction does not show any significant peroxidase activity [19] indicating that microsomal peroxidase activity is not contributed by the contaminated mitochondria. Thus two peroxidase activities studied here are contributed by two different enzymes.

DISCUSSION

The present study indicates that MMI has a direct activating role on the parietal cell to stimulate acid secretion by some intracellular mechanism. We have shown earlier that MMI can directly stimulate acid secretion *in vitro* in frog gastric mucosa in Ussing chamber experiment, even after

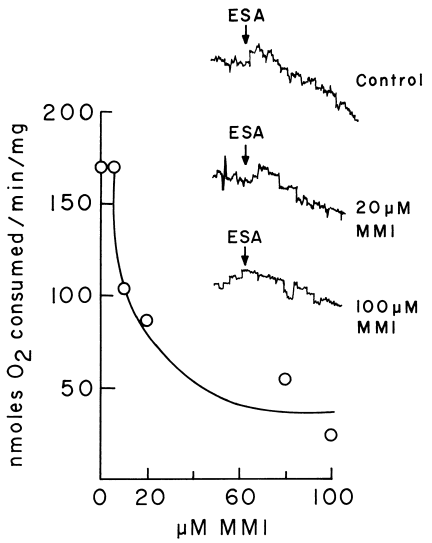

FIG. 6. Effect of varying concentrations of MMI on PG synthetase COX activity of the microsomal fraction of F_3 cells. O_2 consumption was monitored in absence or presence of varying concentrations of MMI as indicated. The inset shows the typical oxygraphic records and the result has been verified by two more experiments.

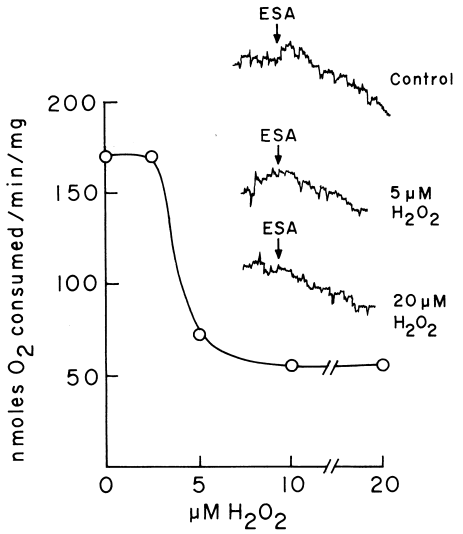

FIG. 7. Effect of varying concentrations of H_2O_2 on the PG synthetase-COX activity of the microsomal fraction of F_3 cells. The procedure was same as described in Fig. 3 except that the microsomal fraction of the F_3 cells were preincubated with H_2O_2 for 5 min before monitoring the O_2 consumption. Inset shows the typical tracing of the O_2 consumption in absence or presence of H_2O_2. This is a representative experiment and has been verified by two more experiments.

addition of histamine to saturate the H_2 receptor [18] indicating that it might work through some intracellular mechanism. MMI effect could also be mimicked with low concentration of H_2O_2 to stimulate acid secretion in isolated rat gastric mucosa [23]. As MMI is a potent

TABLE 5. PG synthetase (peroxidase) activity of microsomal fraction of F_3 cells from mouse stomach

	nmol adrenochrome/min/mg
F_3	458 ± 48
F_3 + MMI 100 μM	20 ± 3
F_3 + H_2O_2 20 μM*	13 ± 2

PG synthetase (peroxidase) activity was measured by the formation of adrenochrome at 480 nm as described under Materials and Methods.
*Preincubated for 5 min.

TABLE 6. MMI-sensitivity to gastric peroxidase and PG synthetase-peroxidase

	IC_{50} MMI	IC_{80-95} MMI
Gastric mucosal peroxidase	7.5 μM	32 μM
Gastric mucosal PG synthetase peroxidase	50 μM	110 μM
F_3 cell peroxidase	7 μM	32 μM
F_3 cell PG synthetase peroxidase	45 μM	100 μM

Assay of GPO activity and PG synthetase peroxidase activity with varying concentrations of MMI has been described in the text. IC_{50} and IC_{80-95} value was determined from the concentration-dependent activity curve.

inhibitor of GPO-catalase system [20, 22, 23], we postulated that the effect might be mediated through increased level of intracellular H_2O_2 which might act like a second messenger [23]. As PG acts as a natural inhibitor of acid secretion [9, 10], it was thought interesting to investigate whether increased acid secretion by MMI *in vivo* is due to decreased biosynthesis of PG by PG synthetase. The present study indicates that MMI itself acts as an inhibitor of the PG-synthetase and the effect can be mimicked by H_2O_2 also. The *in vitro* studies also indicate that MMI is a potent inhibitor of the peroxidase activity of the F_3 fraction enriched in parietal cell. Thus increased accumulation of intracellular H_2O_2 following peroxidase inactivation by MMI is a possibility. Because catalase activity in the parietal cell is very low and is also inactivated by MMI [23], the accumulated H_2O_2 would not be easily metabolized and may thus affect the cellular function. As PG synthetase is a hemoprotein having heme as a prosthetic part and showing intrinsic peroxidase activity [33], it is more likely that MMI might interact close to the heme moiety to show inactivation as previously shown for GPO [22] or lactoperoxidase [34]. The mechanism of inhibition of the COX activity of the PG synthetase is not clear yet. Hydroperoxides have been suggested to inhibit the PG synthetase activity [35–37]. H_2O_2 may also block the COX activity of this enzyme, as shown in cultured porcine aortic endothelial cells [38]. Production of hydroxyl radical ($OH^{\cdot}$) from H_2O_2 by metal-catalyzed Haber–Weiss reaction [39] may also be involved in the inactivation of COX by H_2O_2 [40]. However, further studies are required to prove this. Whatever the mechanism of inactivation of the PG synthetase might be, H_2O_2 is also able to stimulate acid secretion not only in isolated gastric mucosa but also in gastric gland preparation [23]. Attempts were made to demonstrate this effect on the isolated parietal cell, but the result was not impressive because the isolated cell is highly susceptible to the damaging action of H_2O_2 when added from outside.

Although H_2O_2 can directly inhibit the PG-synthetase activity of the parietal cell, its possible role in intracellular Ca^{2+} mobilization [41] or activation of the protein kinase C [42] to stimulate the intracellular signal transduction mechanism for acid secretion cannot be excluded. Evidence is accumulating on the role of intracellular H_2O_2 in the regulation of various cellular functions [43–46]. Moderate concentration of intracellular H_2O_2 and cellular redox state has significant role in gene expression and post-translational modification of proteins [47]. A new concept is emerging that H_2O_2 may act as a signal transduction messenger and affect the critical steps in the signal transduction cascade thereby affecting the basic events of cellular regulation [42]. It is thus more likely that MMI-induced acid secretion is caused at least partially through the elevation of intracellular H_2O_2 affecting the signal transduction mechanism of the parietal cell.

We have shown earlier that MMI stimulates acid secretion *in vivo* through the liberation of histamine [18], which is sensitive to cimetidine [17]. As histamine-stimulated acid secretion should occur at an optimum rate with concominant removal of the inhibitory influence of G_i protein on the adenyl cyclase system and regulated by PG [8–10, 48], it was thought interesting to investigate whether MMI can inhibit the PG-synthetase activity; inhibit PG biosynthesis, and stimulate acid secretion by liberated histamine [18]. Our studies indicate that all the cell fractions isolated from stomach contain the PG synthetase although the activity is high in F_2 and F_3 fractions. PG synthetase is an ubiquitous enzyme. Its high activity in the parietal cell-enriched fraction (F_3) is expected and the endogenous PG will be suitable to modulate the H_2-receptor-stimulated adenylate cyclase activity through the G_i protein. PG apparently binds to the G_i protein causing a decrease in the adenylate cyclase activity through the dissociation of G_i from the heterotrimer $G_i\alpha\beta\gamma$ [49]. Thus G_i activation leads to decreased adenylate cyclase activity controlling cAMP synthesis. Our studies indicate that MMI itself inhibits the PG-synthetase activity probably by interacting with its peroxidase part. If the peroxidase part is inactivated, there will be a build-up of 15-hydroperoxide of the PGG_2 [25] as an intermediate which will inactivate the enzyme and inhibit PG synthesis. Thus after administration of MMI, the inhibitory influence of PG on the adenylate cyclase system should be withdrawn leading to increased acid secretion by the liberated histamine by MMI [18]. We suggest that MMI stimulates acid secretion *in vivo* partially through the activation of the H_2 receptor by the release of histamine [18] with concurrent inhibition of the PG-synthetase activity as well as through the inhibition of the GPO-catalase system [23] leading to the accumulation of intracellular H_2O_2. As H_2O_2 itself can also inactivate the PG-synthetase, it appears that MMI either directly or indirectly through the higher level of endogenous H_2O_2 inactivates the PG synthetase *in vivo* and stimulates acid secretion through decreased biosynthesis of PG. However, inhibition of PG-synthetase activity by MMI may not be the cause of activation of the parietal cell by MMI *in vivo* as observed by increased O_2 consumption. This direct activation may be explained due to possible elevation of the intracellular H_2O_2 following inhibition of the peroxidase activity of the parietal cell. Endogenous H_2O_2 may stimulate signal transduction mechanism presumably through intracellular Ca^{2+} mobilization [41] or activation of the protein kinase C [42]. However, this is a speculation and should be substantiated by direct measurement of the intracellular H_2O_2 under the influence of MMI. Works are in progress for direct measurement of the intracellular H_2O_2 and its possible role in the activation of the parietal cell through signal transduction mechanism.

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