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DISSOCIATION OF GASTRIC ACID AND PEPSINOGEN SECRETION IN RESPONSE TO MERCAPTOMETHYLIMIDAZOLE—A NEW SECRETORY COMPOUND

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Abstract—Mercaptomethylimidazole (MMI), a potent antithyroid drug of the thionamide group, induces both acid and pepsinogen secretion independently in control and pylorus ligated mice. The effect is dose dependent and the drug is more effective than histamine, carbachol or isoproterenol when administered by an intraperitoneal route. MMI-stimulated pepsinogen secretion could be dissociated from the acid secretion by the use of cimetidine and omeprazole which effectively block the acid secretion without affecting the pepsinogen output. Neither acid nor pepsinogen secretion by MMI is inhibited by atropine indicating a lack of muscarinic receptor involvement in both of the processes. Nifedipine and verapamil, the calcium antagonists, by inhibiting the MMI-induced acid secretion can also dissociate pepsinogen secretion from the acid secretion. Clonidine, an α_2-agonist, and hexabarbital, a membrane active barbiturate, also inhibit acid secretion without affecting the pepsinogen output. These data indicate that MMI induces pepsinogen secretion independent of acid secretion. Furthermore, MMI-stimulated acid secretion is not additive with that of the histamine indicating same site (H_2-receptor) of action while its synergistic effect in presence of carbachol (muscarinic receptor) indicates different site of interaction of the two compounds. On the other hand, an additive effect of MMI and carbachol on pepsinogen secretion indicates that while the carbachol effect is mediated through the muscarinic receptor, MMI stimulates pepsinogen secretion through some still unknown mechanism.

Most of the *in vivo* studies indicate that pepsinogen secretion occurs in parallel with acid secretion whenever the latter is stimulated. It is known that gastric acid and pepsinogen secretion originates from different cell types. Acid secretion is mediated via the ATP-driven proton pump (H^+-K^+-ATPase) in the tubulovesicles of the parietal cell [1] whereas pepsinogen secretion from the chief cell follows the typical pathway of secretory protein such as intracellular synthesis, storage in granular compartment and exocytosis of the secretory granules at the luminal plasma membrane [2]. There is evidence that morphological separation of acid and pepsinogen secretion is reflected by a different sensitivity to stimulants and inhibitors. For example, histamine stimulates acid secretion but not the pepsinogen secretion, while isoproterenol stimulates pepsinogen but not the acid secretion [3]. Similarly thiocyanate inhibits acid secretion but has no effect on 8-Br-cAMP or carbachol induced pepsinogen secretion [4]. Again omeprazole inhibits basal, dibutyryl cAMP and histamine stimulated acid secretion but induces pepsinogen release [5]. Although similarities between the two secretory processes such as stimulation with cholinergic agents or with peptide hormones [3, 6, 7] and involvement of Ca^{2+} and cAMP as intracellular messengers [3, 8, 9, 10] do exist, the *in vitro* studies [3–5] indicate that both the processes are dissociable although they may take place simultaneously *in vivo*. However, very little information is available yet on the dissociation of these two secretory processes in intact animal. Recently we have identified a new secretory drug, mercaptomethylimidazole (MMI), which induces both hydrochloric acid and pepsinogen secretion from mouse stomach *in vivo* [11]. We have further observed that MMI induces gastric acid secretion through involvement of the H_2-receptor of the parietal cell [11]. In the present study, we present evidence showing that MMI induces pepsinogen secretion at least not through the cholinergic pathway but through a still unknown mechanism. Moreover, evidence is presented showing that both acid and pepsinogen secretion induced by this new secretory compound could be effectively dissociated *in vivo* with the help of some antisecretory compounds. We suggest that MMI stimulates both acid and pepsinogen secretion through an independent mechanism.

MATERIALS AND METHODS

Materials. Mercaptomethylimidazole (1-methyl-2-thiolimidazole), thiouracil, imidazole, verapamil, nifedipine, clonidine, histamine, carbachol, isoproterenol, cimetidine and pepsin were procured from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and thiourea from Merck (Darmstadt, F.R.G.). Atropine was a product of Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Omeprazole was a gift from Dr W. Beil of Medizinische Hochschule Hannover, F.R.G. Pirenzepine was kindly donated

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Methods. Gastric fluid from mice was collected as described previously [11]. Male Balb-c mice (20–25 g) were fasted for 20 hr and then administered with the chosen drug. The animals were killed by cervical dislocation at the time stipulated, the abdomen was opened and the gastric fluid was collected by flushing the stomach cavity with 2 mL of 0.9% saline. It was centrifuged at 5000 *g* for 10 min in a RC 5B refrigerated Sorvall Centrifuge. The clear supernatant was collected and the volume was recorded.

Pylorus ligation. The experiment was also carried out in pylorus ligated mice to block the loss of gastric fluid if any, and also to prevent contamination from intestinal secretion. This was done in mice according to Shay *et al.* [12] with some modification [11]. The animals were anesthetized with hexabarbital (1.5 mg), the abdomen was opened and the pylorus was ligated. After 2.5 hr when the animals overcame operational shock, the drug was injected intraperitoneally and after a further 2.5 hr the animals were killed by cervical dislocation to collect the gastric juice as described above.

Quantitation of HCl *secreted.* Quantitation of HCl secreted was performed as described earlier [11]. In short, an aliquot (1 mL) of the gastric secretion, after centrifugation, was titrated with 1 mM NaOH to pH 6.5 using an automatic buret and pH stat from Radiometer (Copenhagen, Denmark).

Assay of pepsin activity. Pepsin activity was assayed as described previously [11]. In brief, an aliquot (0.1 mL) of the gastric secretion, after centrifugation was assayed according to the method of Anson and Mirsky [13] as modified by Schlamowitz and Peterson [14]. The pepsinogen secreted in the gastric fluid was activated to pepsin at room temperature for 20 min in the presence of 0.02 N HCl and 0.06 M KCl at pH 2 before assay of pepsin activity. The activity was expressed as units of pepsin activity determined from a standard curve using porcine pepsin as standard. The result was presented as the total unit of pepsinogen secreted as recovered in the total volume of the gastric washing in saline.

Assay of lactic dehydrogenase activity. Lactic dehydrogenase activity was assayed according to the method of Kornberg [15]. An aliquot of the gastric secretion after centrifugation was added to the reaction mixture containing 0.01 M sodium pyruvate, 0.002 M NADH, and 0.1 M potassium phosphate buffer (pH 7.4) and the decrease of extinction at 340 nm was monitored for 1 min at an interval of 10 sec as lactic dehydrogenase activity.

Assay of DNA content of the gastric fluid. DNA content of the gastric fluid was determined according to the method of Stumpf [16]. Briefly DNA was extracted from the stomach fluid as described by Schmidt and Thannhauser [17] and the color developed by cysteine–HCl and H_2SO_4 was measured at 490 nm. The amount of DNA was determined from a standard curve.

Dose and vehicle of the drugs. All the drugs used were in aqueous solution except omeprazole which was dissolved in normal saline containing 25% ethanol just before injection. Nifedipine was prepared in absolute ethanol and was protected from light. The volume of the drug injected was usually 0.1 to 0.2 mL by an intraperitoneal route and in all cases the same volume of the vehicle was administered to the control mice. Dose–response with MMI was studied by intraperitoneal administration of the drug from 0.275 mg to 2.2 mg and collecting the gastric secretion at 2.5 hr. Duration profile of the gastric secretion was studied over a period of 4 hr after administration of the optimum dose (0.55 mg) of MMI. The effectiveness of each of the secretory compounds was compared with MMI at its optimum dose and optimum time of gastric secretion predetermined from the dose–response and duration profile experiments. To study the effect of antisecretory compound, it was injected intraperitoneally 30–60 min prior to the administration of the secretory drug.

Statistical evaluation. All the data presented are mean ± SE (standard error of the mean). Significance was calculated from Student's *t*-test.

RESULTS

Administration of mercaptomethylimidazole to both pylorus ligated and nonligated animals induces a copious flow of gastric secretion rich in hydrochloric acid and pepsinogen. The volume of the gastric juice accumulated both in pylorus nonligated and ligated animals was about 6-fold over the control value (Table 1). These values are however apparent because of the possibility that some gastric juice might be lost due to gastric emptying in nonligated stomach, while in ligated stomach secretion may be reduced due to surgical procedure. This might explain why there was no significant difference in the volume of gastric secretion between ligated and nonligated animals. Administration of MMI to both pylorus nonligated and ligated animals led to the accumulation of a 9- and 6-fold higher amount of HCl over the control value respectively. MMI also caused 8-fold stimulation of pepsinogen secretion in the pylorus nonligated animals. The response was strikingly different in pylorus ligated animals where the control pepsinogen secretion was stimulated nearly 7-fold over the nonligated control value. This stimulation may either be due to a block of the loss of gastric fluid resulting from pylorus ligation or may be due to various factors involved during surgical ligation of the pylorus. However, this value was further increased more than 3-fold after MMI administration. Since MMI response to both acid and pepsinogen secretion is much better in unoperated control than pylorus ligated animals, control mice could be conveniently used for the rest of the experiments. Furthermore, pylorus ligation has some disadvantages in that other factors like anesthesia, vasovagal reflex, altered secretion of gastrointestinal hormones and operational stress come into play as discussed earlier [11, 18].

In order to find out whether both acid and pepsinogen secretion in response to MMI could be dissociable or not, dose–response as well as the duration profile of both the secretions were investigated. The dose–response curves as shown in Fig. 1 indicate that both acid and pepsinogen secretion were linearly stimulated by MMI up to a dose of 0.55 mg above

Table 1. Effect of MMI on gastric acid and pepsinogen secretion in control and pylorus ligated mice

	Pylorus nonligated			Pylorus ligated		
	Volume secreted (μL)	HCl secreted (μmol)	Pepsinogen secreted units	Volume secreted (μL)	HCl secreted (μmol)	Pepsinogen secreted units
Control	46 ± 6	2.3 ± 0.15	44.4 ± 3.14	63 ± 14	3.05 ± 0.86	293.8 ± 22.7
N	12	118	124	19	13	13
Control + MMI	310 ± 25*	20.6 ± 1.2*	362.5 ± 14.4*	340 ± 38*	17.1 ± 2.7*	961.2 ± 83.5*
N	15	49	75	24	13	15

Pylorus ligation was made as described under Materials and Methods. An amount 0.55 mg MMI in 0.1 mL water was injected intraperitoneally into each of the pylorus nonligated and pylorus ligated mice while the control group received 0.1 mL of water as vehicle. The animals were killed after 2.5 hr to collect the gastric secretion. Estimation of HCl and pepsinogen secreted was described in the text.

* $P < 0.001$.

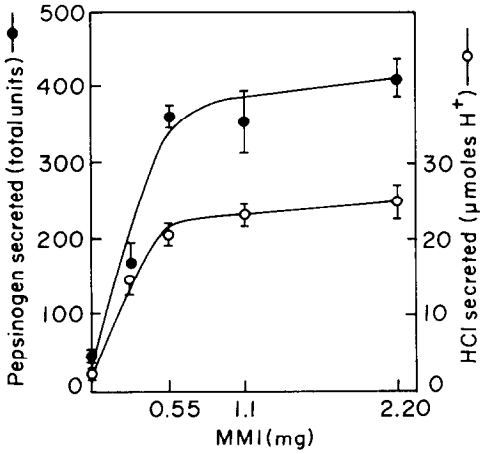

Fig. 1. Dose response profile of acid and pepsinogen secretion after administration of MMI. The gastric secretion was collected by washing the stomach content with 2 mL of 0.9% saline 2.5 hr after intraperitoneal administration of varied amount of MMI as indicated. N = 12–124.

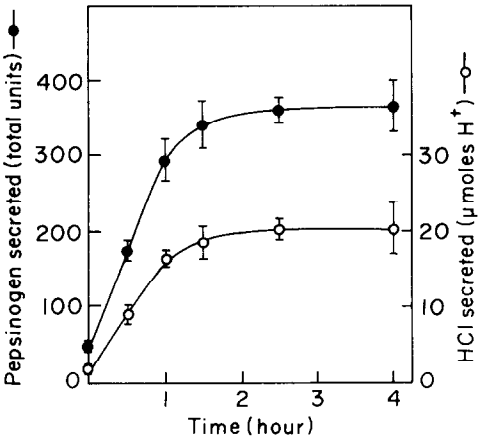

Fig. 2. Duration profile of gastric acid and pepsinogen secretion after administration of MMI. The procedure was the same as in Fig. 1 except that gastric secretion was collected at various time periods as indicated after administration of 0.55 mg MMI. N = 12–124.

which both the response tends to saturate at least up to a dose of 2.2 mg. The duration profile of both the secretions with the optimum dose of 0.55 mg was shown in Fig. 2. The result shows that acid secretion takes place almost linearly up to 1 hr after which the rate of secretion tends to saturate and above 1.5 hr the acid secretory rate showed a plateau. The duration profile of the pepsinogen secretion in response to MMI also shows similarity with that of acid secretion. Thus no significant dissociation of the secretion of acid and pepsinogen in response to MMI is evident from the dose–response or duration profile experiment.

Since MMI is an antithyroid drug of thionamide group having similar stimulatory effect on both acid and pepsinogen secretion, it was thought interesting to investigate whether similar or differential response on these secretory processes could be obtained with other antithyroid drugs of the same family. Table 2 shows that thiourea caused about a 2.5-fold stimulation of both acid and pepsinogen secretion and is much less active than MMI. Thiouracil, on the other hand, showed a differential response on these two secretions. While it has no effect on acid output, it stimulates pepsinogen secretion by 2.5-fold. Similarly, imidazole which is of course not an antithyroid drug, also enhances pepsinogen secretion without affecting the acid secretion. Thus MMI appears to be the most effective of the drugs of this group tested in stimulating both acid and pepsinogen secretion.

The effectiveness of MMI in inducing secretion of acid and pepsinogen was compared with that of the known secretagogues such as histamine, isoproterenol and carbachol. Table 3 shows that MMI increased secretion of both acid and pepsinogen to a greater extent than histamine when administered by an intraperitoneal route. Although histamine is known as a specific secretagogue for gastric acid secretion, it also stimulates pepsinogen secretion 2-fold in mice. We cannot afford any explanation how histamine stimulates pepsinogen secretion. However, washout of some already trapped pepsinogen in the gastric pits by the output of acid gastric

Table 2. Effect of various antithyroid drugs on gastric acid and pepsinogen secretion

	HCl secreted (μmol)		Pepsinogen secreted units	
		N		N
Control	2.3 ± 0.15	118	44.4 ± 3.14	124
Control + MMI	20.6 ± 1.2†	49	362.5 ± 14.4†	75
Control + thiourea	5.66 ± 0.92*	13	100.7 ± 14†	13
Control + thiouracil	2.26 ± 0.51 NS	14	116.8 ± 15.6†	23
Control + imidazole	2.0 ± 0.57 NS	12	101.5 ± 10.7†	11

Gastric secretion was collected 2.5 hr after intraperitoneal administration of 0.1 mL (0.55 mg) of the compound indicated for estimation of HCl and pepsinogen secreted.
* $P < 0.05$, † $P < 0.001$, NS not significant.

Table 3. Effect of MMI in stimulating gastric acid and pepsinogen secretion in comparison to other secretagogues

	HCl secreted (μmol)		Pepsinogen secreted units	
		N		N
Control	2.3 ± 0.15	118	44.4 ± 3.14	124
Control + MMI	20.6 ± 1.2†	49	362.5 ± 14.4†	75
Control + histamine	5.23 ± 1.35*	12	98.8 ± 11.9*	22
Control + isoproterenol	1.25 ± 0.44*	11	133.3 ± 18.1†	11
Control + carbachol	4.32 ± 0.88*	9	150.2 ± 12.4†	14

Mice were administered intraperitoneally with MMI (0.55 mg), histamine (0.55 mg), carbachol (0.1 μg) or isoproterenol (5 μg). Gastric secretion was collected after 2.5 hr in all cases except for isoproterenol and carbachol which were collected after 1 hr. The dose and time were predetermined for optimum secretion in each case.
* $P < 0.05$, † $P < 0.001$.

juice may be a possibility. Isoproterenol, the β-adrenergic agonist, known to stimulate specifically the pepsinogen secretion, also has the same effect in mice at the optimum dose of 5 μg but inhibits the acid secretion to some extent. However, when compared, MMI was also found to stimulate pepsinogen secretion to a greater extent than isoproterenol. Carbachol, the well-known cholinergic agent for both acid and pepsinogen secretion was found to be active at a dose of 0.1 μg in stimulating both acid and pepsinogen secretion but, when compared, it is less active than MMI. The stimulatory effect of carbachol decreases above a dose of 0.1 μg and at a very high dose of 15 μg, carbachol almost blocks the basal acid secretion without affecting the basal pepsinogen output (data not shown).

With a view to finding out the possible mechanism of gastric acid and pepsinogen secretion induced by MMI as well as to ascertain whether both the secretions could be dissociable or not, the effect of some antisecretory compounds were investigated as shown in Table 4. Cimetidine, the well-known H_2-receptor antagonist, significantly inhibited the MMI-induced acid secretion but had no effect on pepsinogen secretion. As both acid and pepsinogen secretion may be stimulated by a common mediator like endogenous acetylcholine which may be liberated by cholinergic stimulation by MMI, the effect of atropine, the anticholinergic agent was studied. The result shows that neither acid nor pepsinogen secretion could be blocked by atropine. Omeprazole which inhibits acid secretion by interaction with the proton-pumping H^+-K^+-ATPase, blocks MMI-induced acid secretion almost completely but has no significant effect on pepsinogen secretion. In order to investigate the role of calcium entry mechanism influencing the MMI effect on acid and pepsinogen secretion, the effect of some calcium antagonists was studied. Nifedipine and verapamil significantly inhibit the MMI-induced acid secretion but they have no significant effect on pepsinogen output. Clonidine, an α_2-agonist, also inhibits acid secretion without affecting the pepsinogen secretion significantly. Similarly, hexabarbital, a known membrane damaging agent, also blocks acid secretion but has no effect on MMI-induced pepsinogen secretion.

Further information about the mechanism of action of MMI on acid and pepsinogen secretion could be obtained when the secretions were studied in the presence or absence of a known agonist. Table 5 shows that MMI when injected with histamine could not show the additive effect on acid secretion, indicating that MMI has no other alternative site of action other than the H_2-receptor on the parietal cells. Similarly pepsinogen secretion by MMI in the presence of histamine did not show any additive effect indicating that their stimulation may share a common mechanism. The possibility of a still unknown receptor for both histamine and MMI on chief cells cannot be excluded. However, MMI when injected with carbachol showed at least an additive effect on both gastric acid and pepsinogen secretion indicating that MMI and carbachol stimulate both acid and pepsinogen secretion through different pathways.

Table 4. Dissociation of gastric acid and pepsinogen secretion with various antisecretory agents

System	HCl secreted (μmol)	N	Pepsinogen secreted units	N
1. Control	2.3 ± 0.15	118	44.4 ± 3.1	124
2. Control + MMI	20.6 ± 1.2*	49	362.5 ± 14.4*	75
3. Control + cimetidine + MMI	5.74 ± 1.22*	12	325.0 ± 71.1 NS	12
4. Control + atropine + MMI	17.72 ± 2.53 NS	13	375.1 ± 19.1 NS	13
5. Control + pirenzepine + MMI	17.52 ± 1.72 NS	13	418.8 ± 32.5 NS	10
6. Control + omeprazole + MMI	3.70 ± 1.02*	9	305.9 ± 36.1 NS	14
7. Control + nifedipine + MMI	12.61 ± 1.76*	16	375.6 ± 36.4 NS	11
8. Control + verapamil + MMI	8.34 ± 1.14*	19	304.1 ± 22 NS	14
9. Control + clonidine + MMI	6.95 ± 0.84*	12	318.0 ± 28.6 NS	12
10. Control + hexabarbital + MMI	5.55 ± 1.61*	11	297.8 ± 31.9 NS	10

A solution of cimetidine (2 mg), atropine (0.2 μg), pirenzepine (0.2 μg), omeprazole (0.2 mg), nifedipine (200 μg), verapamil (200 μg), clonidine (20 μg) or hexabarbital (1.5 mg) in 0.1 mL was injected (i.p.) to each animal of the respective group 0.5–1 hr before the administration of 0.1 mL (0.55 mg) of MMI (i.p.). Animals were killed 2.5 hr after MMI injection to collect the gastric secretion for HCl and pepsinogen estimation. For statistical evaluation of both acid and pepsinogen secretion, system 2 is compared with system 1 and systems 3 to 10 are compared with system 2.

* $P < 0.01$, † $P < 0.001$, NS not significant.

Table 5. Effect of MMI on gastric acid and pepsinogen secretion in presence or absence of histamine or carbachol

System	HCl secreted (μmol)	N	Pepsinogen secreted units	N
1. Control	2.3 ± 0.15	118	44.4 ± 3.14	124
2. Control + MMI	16.54 ± 1.17‡	22	295.5 ± 27.5‡	24
3. Control + histamine	6.85 ± 1.62†	12	90.1 ± 19.7*	12
4. Control + MMI + histamine	16.46 ± 2.26 NS	13	311.0 ± 29.1 NS	14
5. Control + carbachol	4.32 ± 0.88*	9	150.2 ± 12.4‡	14
6. Control + MMI + carbachol	23.19 ± 2.08†	17	454.06 ± 24.1‡	16

A quantity of 0.1 mL of a solution of MMI (0.55 mg), histamine (0.55 mg) or carbachol (0.1 μg) was injected to each animal of the respective group either singly or in combination as indicated. Gastric secretion was collected after 1 hr for estimation of acid and pepsinogen secreted. For statistical evaluation of both acid and pepsinogen secretion, systems 2, 3 and 5 are compared with system 1 while system 4 is compared with systems (2 + 3) and system 6 is compared with systems (2 + 5).

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

DISCUSSION

Mercaptomethylimidazole appears to be one of the best secretory drugs when tested by an intraperitoneal route of administration and could be very conveniently used *in vivo* for the study of both acid and pepsinogen secretion in control and pylorus ligated mice. It is possible that MMI appears to be a better secretory compound than histamine, carbachol or isoproterenol because MMI is either not readily metabolized or the latter compounds are metabolized to some extent before reaching the stomach when administered intraperitoneally. However *in vitro* experiments on isolated cells will establish which one is the most effective as a secretory compound.

The mammalian secretion of both acid and pepsinogen *in vivo* are difficult to dissociate though acid is being secreted from the parietal cells actively while the other is from the chief cells after intracellular synthesis, storage and exocytosis. Dissociation of acid and pepsinogen secretion has been reported in *in vitro* experiments [10, 19, 20] in the recent past. However, very little information is available on the dissociation of these two secretory processes in intact animals. Our studies show that the increase in pepsinogen secretion due to pylorus ligation in mice is not dependent on acid secretion as the latter is not significantly elevated due to pylorus ligation. This is in contrast to rats where pylorus ligation leads to stimulation of acid secretion possibly through the vaso-vagal reflexes [21]. Thiouracil and imidazole do not augment gastric acid secretion but stimulate pepsinogen release indicating that the latter is not dependent on acid secretion. This effect is comparable to that of isoproterenol which is known to specifically induce pepsinogen secretion without affecting acid secretion [3]. However, exogenous isoproterenol was found to be inhibitory to acid secretion in mouse as has been reported earlier [22, 23]. Another interesting finding is that histamine not only induces acid secretion but also stimulates pepsinogen output in mouse contrary to the effect

observed in rabbit [3] or dog [24]. The most significant observation of the present investigation is that both acid and pepsinogen secretion induced by MMI could be effectively dissociated with the help of some antagonists which also help to suggest the possible mechanism of action of MMI on acid and pepsinogen secretion. Sensitivity of MMI-induced acid secretion to cimetidine and omeprazole but insensitivity of pepsinogen secretion not only indicates that pepsinogen secretion by MMI is independent of acid secretion, but also suggests that MMI induces acid secretion through the involvement of H_2-receptor as well as through the proton pumping H^+-K^+-ATPase of the parietal cells. However, failure of atropine to block either of the processes suggests the absence of a cholinergic pathway in both the secretions induced by MMI. Pirenzepine, which is less potent than atropine in blocking the carbachol stimulated pepsinogen secretion [25], also has no effect indicating lack of involvement of the M_1-cholinergic pathway in either of the processes. Calcium antagonists such as nifedipine and verapamil can also dissociate acid and pepsinogen secretion. Partial blocking of MMI-induced acid secretion by calcium antagonist indicates partial involvement of a calcium entry mechanism which is absent in MMI-induced pepsinogen secretion. The role of the calcium antagonist is however still controversial. A large number of workers have shown that secretagogue-stimulated acid secretion is at least partially mediated through calcium entry mechanisms [8, 26–29]. It has been reported that most of the calcium channel blockers act as nonspecific inhibitors of H^+-K^+-ATPase [30] while Chew [31] and Chew and Brown [9] have shown that calcium antagonists inhibit acid secretion nonspecifically and parietal cells respond to secretagogues through the increase in intracellular calcium released from some intracellular source. Similarly, secretagogue-induced pepsinogen secretion appears to be mediated through cAMP [3, 32, 33] or through intracellular calcium [10, 34]. Recently it has been suggested that modulation of adenyl cyclase by change of intracellular calcium may play a role in the potentiation of pepsinogen secretion by some secretagogues [35]. Since MMI-induced pepsinogen secretion is not affected by H_2-receptor antagonists or by anticholinergic agents, its effect may be mediated through some mechanism which remains to be investigated. However, the possibility that MMI is inducing pepsinogen secretion through some unidentified receptor on the chief cells cannot be excluded. Although clonidine can effectively dissociate pepsinogen secretion from acid secretion, its mechanism of inhibition of MMI induced acid secretion is poorly understood. Clonidine is known to stimulate the α_2-receptor [36] and decreases the vagally mediated discharge of acetylcholine [37] thereby blocking acid secretion. As the MMI effect is mediated through the H_2-receptor, it is not clear how clonidine inhibits this process. The mechanism of action of MMI on acid and pepsinogen secretion has further been clarified by studying the phenomena in the presence of some agonists. The additive effect of carbachol but not of histamine on acid secretion is consistent with interaction of MMI with the H_2-receptor of the parietal cells. An additive effect of MMI, in the presence of carbachol on pepsinogen secretion, further suggests that while the carbachol effect is mediated through specific muscarinic receptor on chief cells, the MMI effect is possibly mediated either through some unknown cell surface receptor or through some intracellular mechanism.

We therefore suggest that although MMI stimulates both acid and pepsinogen secretion simultaneously in a similar way, pepsinogen secretion is not due to its primary effect on acid secretion or by simple washing out of the existing pepsinogen in the gastric pits by acid output. Pepsinogen secretion is not due to any damage of the chief cells as evidenced by the absence of any significant increase in DNA in the gastric juice after MMI administration (data not shown). Assay of lactic dehydrogenase activity in the gastric secretion could not be used as a marker for cell damage in *in vivo* experiments as it was found to be inactivated at acid pH of the gastric secretion (data not shown). Cholinergic pathway is not involved in either of the secretory processes induced by MMI. While acid secretion is mediated through the involvement of the H_2-receptor of the parietal cells, pepsinogen secretion is possibly mediated either through potentiation of some intracellular mechanism related to pepsinogen synthesis, its storage in granules and exocytosis or through some extracellular receptor on the chief cells.

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