Biochemical Pharmacology, Vol. 33, No. 22, pp. 3621–3625, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

DIFFERENCES IN THE INTERACTION OF HISTAMINE H_2 RECEPTOR ANTAGONISTS AND TRICYCLIC ANTIDEPRESSANTS WITH ADENYLATE CYCLASE FROM GUINEA PIG GASTRIC MUCOSA

BIE SHUNG TSAI* and TOBIAS O. YELLIN†
Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897, U.S.A.

(*Received* 23 *November* 1983; *accepted* 14 *March* 1984)

Abstract—The interaction of adenylate cyclase with histamine H_2 receptor agents and with tricyclic antidepressants was studied in guinea pig gastric mucosal membranes. The H_2 receptor antagonist tiotidine acted as a competitive inhibitor of histamine-stimulated adenylate cyclase. The tricyclic antidepressants imipramine and amitryptyline were also competitive inhibitors. The dissociation constant of imipramine was the same whether histamine or dimaprit was used to activate the enzyme. In membrane preparations that had been stored frozen, there was a marked increase in the concentration of histamine or dimaprit required to cause half-maximal enzyme stimulation, and the dissociation constants of some classical H_2 receptor antagonists were greatly increased. In contrast, the dissociation constants of the antidepressants were either unchanged or decreased. These results suggest that antidepressants are potent blockers of H_2 receptors in gastric mucosal membranes, but there are differences between antidepressants and classical H_2 receptor antagonists in their interaction with H_2 receptors.

Histamine plays a major role in the physiological control of gastric acid secretion [1, 2]. The histamine receptors in gastric mucosa are pharmacologically classified as H_2 receptor subtype [3, 4] and are coupled to adenylate cyclase [4–9]. Cyclic AMP is thought to mediate the action of histamine on gastric acid secretion [4, 10].

Antagonists of histamine H_2 receptors such as cimetidine have been proven clinically to be effective in the inhibition of gastric acid secretion and in the treatment of gastric ulcer [11, 12]. Tricyclic antidepressants have also been shown to be effective in the prevention of ulcer formation in animals [13–15]. However, in *in vivo* studies, they appeared to be weak inhibitors of histamine-stimulated gastric acid secretion [16–18]. Recently, tricyclic antidepressants have been reported to be potent antagonists of histamine H_2 receptors in the brain [19, 20]. In the present study, we have investigated the activity of tricyclic antidepressants on H_2 receptors coupled to adenylate cyclase in fundic gastric mucosal membranes. For the purpose of comparison, the effects of classical H_2 antagonists on this system were also studied.

MATERIALS AND METHODS

Membrane preparation. Male guinea pigs weighing 300–550 g were used for the isolation of fundic gastric mucosa. The mucosal tissues were prepared according to the method previously described [21]. After the guinea pigs were killed by cervical dislocation, the fundic part of the stomach was removed and washed thoroughly with ice-cold saline. The stomachs were then rinsed several times with ice-cold Tris buffer solution (0.15 M sucrose, 5 mM Tris, 3 mM $MgCl_2$, and 1 mM EDTA, pH 7.4) and blotted with Whatman filter paper. The mucosal tissue was scraped from the stomach walls and homogenized for five to six strokes with a Teflon–glass homogenizer, using a ratio of 1 g of tissue to 3 ml of Tris buffer solution. The homogenate was filtered through a nylon mesh screen (No. 114T from Nytex) and centrifuged at 3000 *g* for 10 min. The pellet was subsequently washed three times by homogenization and centrifugation in 10 ml of Tris buffer without sucrose. The final pellet was rehomogenized with five strokes in 3.5 ml of ice-cold Tris buffer solution without sucrose. Adenylate cyclase activity was determined in freshly-prepared membranes and in membranes that had been rapidly frozen with dry ice in acetone and stored at −80° for up to 2 weeks before thawing.

Adenylate cyclase assay. Adenylate cyclase was assayed according to the method previously described [21]. The assay mixture (pH 7.4) contained 50 μl of Tris–HCl, 40 mM; $MgCl_2$, 4.8 mM (in excess of EDTA, 0.2 mM); cAMP, 0.1 mM; ATP, 0.1 mM; [α-^{32}P]ATP, 1–2 × 10^6 cpm; creatine phosphate, 5 mM; and creatine-phosphate kinase, 2 units. Incubations were carried out for 9 min at 37° and terminated by adding 1 ml of a solution containing [^{3}H] cAMP (15,000 cpm), 100 μg ATP and 50 μg cAMP. The enzyme reaction was linear with the protein concentration used (30–70 μg). Enzyme activity was

* Author to whom all correspondence should be sent. Present address: Searle Research and Development, Division of G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077.

† Present address: Beckman Instruments, Inc., Bioproducts Operations, Palo Alto, CA 94304.

also linear in the presence or absence of histamine for at least 12 min. Addition of H_2 antagonists or tricyclic antidepressants produced a prompt inhibition of histamine-stimulated adenylate cyclase activity. Data shown represent the mean ± S.E.M. of at least three experiments, each with a different membrane preparation. All assays were performed in triplicate or quadruplicate. Protein was determined by the method of Lowry *et al.* [22].

Calculation of the apparent dissociation constants of H_2 receptor antagonists. The apparent dissociation constants (K_D) of the antagonists were estimated using the following dose-ratio equation [23]:

$$K_D = \frac{[\text{antagonist}]}{\text{DR-1}}$$

where [antagonist] refers to the concentration of antagonist used. DR (the dose-ratio) is the ratio of agonist concentrations needed to produce 50% of maximal stimulation of adenylate cyclase in the presence or absence of antagonists. H_2 receptor antagonists and antidepressants were found to inhibit basal adenylate cyclase activity generally by less than 20%. This inhibition of basal enzyme activity by classical H_2 receptor antagonists and antidepressants was due to a very small amount of endogenous histamine (~0.2 μM, unpublished observation) present in the membrane preparations. This inhibited basal enzyme activity, which can be considered as the true basal enzyme activity, was used for calculating the stimulation of adenylate cyclase caused by agonists. The stimulation of adenylate cyclase by agonist was then expressed as a percentage of maximal stimulation obtained with that agonist. The unpaired Student's *t*-test was used for statistical analysis.

Materials. Histamine dihydrochloride was purchased from the Fisher Scientific Co., and [^{3}H]cAMP and [α-^{32}P]ATP from New England Nuclear. Dimaprit HCl, metiamide, and cimetidine were gifts from Smith, Kline & French Laboratories. Adenosine 3′:5′-cyclic monophosphoric acid, adenosine 5′-triphosphate, phosphocreatine, creatine phosphokinase, and imipramine were obtained from the Sigma Chemical Co. 5′-Guanylyl-imidodiphosphate (GppNHp) was purchased from ICN. Amitriptyline and iprindole were supplied by Merck Sharp & Dohme Research Laboratory and Wyeth Laboratories respectively.

RESULTS

Effects of H_2 receptor antagonists and tricyclic antidepressants on fresh membranes. Histamine stimulation of adenylate cyclase is shown in Fig. 1. Half-maximal stimulation occurred at 0.8 to 1.2 μM (N = 12) and maximal stimulation was reached around 0.01 or 0.1 mM. Tiotidine (0.4 μM), which is a competitive antagonist of H_2 receptor in gastric mucosa [24, 25], markedly shifted the dose–response curve of histamine to the right. Imipramine (3 μM) also caused the dose–response curve of histamine to shift to the right, but to a lesser extent than that produced by tiotidine. The apparent dissociation constants of tiotidine and imipramine were 0.019 ± 0.004 and 0.60 ± 0.11 μM respectively.

To determine whether the inhibition of histamine-

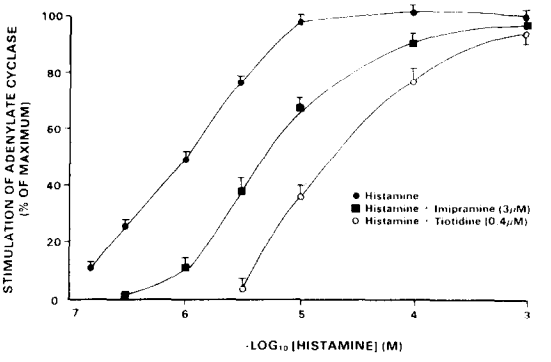

Fig. 1. Effects of tiotidine and imipramine on the dose–response curve of histamine activation of adenylate cyclase from freshly prepared fundic gastric mucosa. The data are expressed as a percentage of the maximal stimulation elicited by histamine and represent the mean ± S.E.M. for three to six experiments on different membrane preparations. Basal and maximal histamine-stimulated adenylate cyclase activities were 20 ± 2.4 and 66 ± 10 pmoles/min/mg protein, N = 6, respectively.

stimulated adenylate cyclase by antidepressants was mediated through H_2 receptors, dimaprit, a specific H_2 receptor agonist [26], was used to stimulate the enzyme. Dimaprit stimulation of adenylate cyclase is illustrated in Fig. 2A. The efficacy of dimaprit to stimulate adenylate cyclase was 85% of that of histamine. Imipramine at 3 μM also inhibited dimaprit-stimulated adenylate cyclase activity. The dissociation constant of imipramine, as determined by the dose-ratio equation, was 0.52 ± 0.07 μM (N = 3) when dimaprit was used as an agonist, a value not significantly different from that calculated when histamine was used to stimulate the enzyme. A Schild plot [27] for inhibition by imipramine of dimaprit-stimulated adenylate cyclase was determined. As shown in Fig. 2B, the slope of the Schild plot is 1.06, which is not significantly different from unity. This suggests that the inhibition by imipramine of dimaprit-stimulated adenylate cyclase was competitive in nature. The dissociation constant of imipramine obtained from the Schild plot was 0.4 μM, a value comparable to 0.52 μM which was derived from the dose-ratio equation. Imipramine and other antidepressants were found not to inhibit NaF- or GppNHp-stimulated enzyme activity.

Effects of freezing and thawing of the membrane preparation on its interaction with H_2 receptor agents and tricyclic antidepressants. During the course of our investigation of adenylate cyclase activity in membranes which had been frozen, a rather unique property of adenylate cyclase, with respect to H_2 receptor agonists and antagonists and tricyclic antidepressants, was unexpectedly revealed. The concentration of histamine and dimaprit required to produce half-maximal enzyme stimulation (EC_{50}) was increased by 5- and 4-fold respectively, in the frozen–thawed membranes (Fig. 3, data for dimaprit not shown). These results suggest that the freezing and thawing processes might have decreased the sensitivity of adenylate cyclase to histamine and dimaprit. The maximal histamine- and dimaprit-stimulated enzyme activity remained the same in

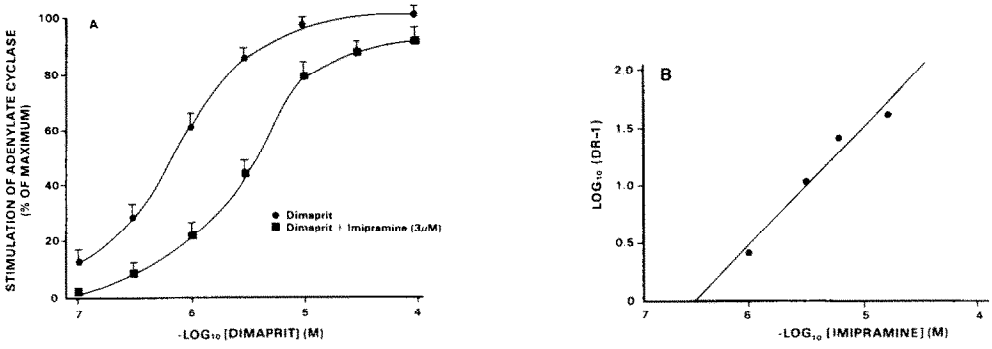

Fig. 2. (A) Effect of imipramine (3 μM) on the dose–response curve of dimaprit activation of adenylate cyclase from freshly prepared fundic gastric mucosa. The data are expressed as a percentage of the maximal stimulation elicited by dimaprit and represent the mean ± S.E.M. for three experiments on different membrane preparations. Basal and maximal dimaprit-stimulated adenylate cyclase activities were 16 ± 1.8 and 46 ± 3 pmoles/min/mg protein, N = 3, respectively. (B) Schild plot of inhibition by imipramine of dimaprit-stimulated adenylate cyclase activity. The dose-ratio (DR) was calculated as described in Methods. The slope (1.06), determined by linear regression analysis ($r = 0.97$), is not significantly different from unity. The dissociation constant of imipramine (the intercept with the abscissa) is 0.4 μM. Each point is the mean of two experiments. The membranes used in these experiments were different from those in Fig. 2A.

both fresh and frozen–thawed membranes. There was a striking decrease in the ability of tiotidine to inhibit histamine-stimulated adenylate cyclase in frozen–thawed membranes (Fig. 3). The dose-dependent stimulation of adenylate cyclase by histamine was shifted 3-fold to the right by tiotidine in frozen–thawed membranes, as compared to a 23-fold shift in fresh membranes. In contrast, the ability of imipramine to inhibit histamine-stimulated adenylate cyclase tended to increase in frozen–thawed membranes (Fig. 3). When the dose–response curve of histamine was studied in the presence of other H_2 antagonists and antidepressants, the results shown in Table 1 were obtained. The ability of metiamide and cimetidine to inhibit histamine-stimulated adenylate cyclase was greatly reduced in frozen–thawed membranes; an approximate 10-fold increase in their dissociation constants were observed. This finding is similar to that seen with tiotidine. The

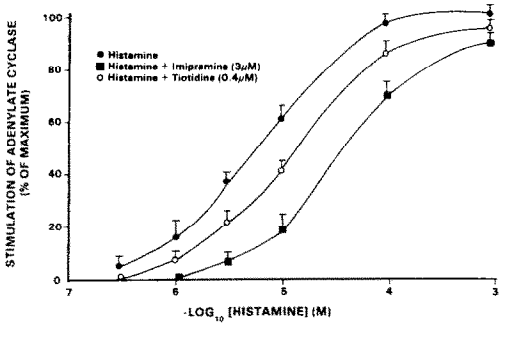

Fig. 3. Effects of tiotidine and imipramine on the dose–response curve of histamine activation of adenylate cyclase from frozen and thawed fundic gastric mucosa. The data are expressed as a percentage of the maximal stimulation elicited by histamine and represent the mean ± S.E.M. for five to six experiments on different membrane preparations. Basal and maximal histamine-stimulated adenylate cyclase activities were 18 ± 2 and 58 ± 6 pmoles/min/mg protein, N = 6, respectively.

dissociation constants of other antidepressants were either unchanged (doxepin, $P > 0.05$) or were reduced significantly (amitryptyline, $P < 0.02$) when comparing results from fresh membrane preparations with those from frozen–thawed preparations.

DISCUSSION

Recent research has shown that tricyclic antidepressants such as imipramine and amitryptyline are potent competitive inhibitors of histamine H_2 receptors in brain homogenates [19, 20]. However, animal studies revealed that they have very little activity on gastric mucosal histamine H_2 receptors in inhibiting gastric acid secretion [16–18, 28]. This discrepancy could be explained if there are different H_2 receptor subtypes in various tissues. For example, the H_2 receptors in the brain may have a high affinity for tricyclic antidepressants, whereas the receptors in the gastric mucosa may have a low affinity for them. Our findings, however, do not support this hypothesis by showing that tricyclic antidepressants were potent competitive inhibitors of histamine H_2 receptors coupled to adenylate cyclase in gastric mucosal membranes, as they are in the brain tissue. Based on our results one would expect that tricyclic antidepressants should potently inhibit histamine-induced gastric acid secretion. This apparent different specificity of H_2 receptors for tricyclic antidepressants, but not for classical H_2 receptor antagonists, observed in intact gastric mucosa and in mucosal membrane preparations, has also been found in brain preparations. Tuong *et al.* [29] reported that the dissociation constants of tricyclic antidepressants for H_2 receptors in guinea pig brain slice preparations are 100 times greater than in brain homogenates, whereas the dissociation constants of classical H_2 receptor antagonists remain the same in brain slices and in brain homogenates. They postulated that the different specificities of brain H_2 receptors for tricyclic antidepressants in intact cells

Table 1. The EC_{50} (H_2 agonists) and the K_D (H_2 antagonists and antidepressants) values determined from fresh and from frozen and thawed adenylate cyclase in fundic gastric mucosal membranes*

	EC_{50} or K_D (μM)	
	Fresh	Frozen
H_2 agonist		
Histamine	1.06 ± 0.1 (6)	4.5 ± 0.28† (12)
Dimaprit	1.04 ± 0.23 (3)	4.0 ± 0.26† (3)
H_2 antagonist		
Metiamide	0.70 ± 0.03 (3)	6.86 ± 1.49† (6)
Tiotidine	0.017 ± 0.004 (3)	0.187 ± 0.03† (5)
Cimetidine	0.68 ± 0.08 (4)	5.4 ± 0.63† (2)
Tricyclic antidepressants		
Imipramine	0.60 ± 0.10 (3)	0.37 ± 0.03‡ (6)
Amitriptyline	0.41 ± 0.06 (3)	0.11 ± 0.03† (3)
Doxepin	0.69 ± 0.20 (3)	0.36 ± 0.08‡ (3)
Atypical antidepressant		
Iprindole	1.98 ± 0.56 (3)	1.29 ± 0.2‡ (3)

* EC_{50} is the concentration of agonist required to produce a half-maximal increase in adenylate cyclase activity. K_D is the apparent dissociation constant of antagonist, which was derived from the dose-ratio equation described in Methods. Values shown are means ± S.E. from the number of separate experiments indicated in the parentheses.

† $P < 0.05$ as compared with values from fresh adenylate cyclase.

‡ $P > 0.05$ as compared with values from fresh adenylate cyclase.

and those in cell-free preparations probably are caused by a change in H_2 receptor conformation induced during the preparation of the homogenates. Recently, Angus and Black [28] have hypothesized that tricyclic antidepressants are less potent in pharmacological assay on isolated cardiac papillary muscle than in biochemical assays on brain homogenates. These investigators attributed the greater potency of the tricyclic antidepressants in the intact tissue to the additional effect of these agents, which might inhibit phosphodiesterase activity, thus increasing intracellular cAMP concentrations and, hence, offsetting the H_2 receptor blockade effected by tricyclic antidepressants. Further studies are needed to clarify these hypotheses.

The major finding of our studies is that, although tricyclic antidepressants were competitive antagonists at H_2 receptors in fundic gastric mucosal membranes, their interaction with H_2 receptors can be distinguished from that of classical H_2 receptor antagonists when dissociation constants were compared in fresh versus frozen membranes. Thus, freezing and thawing selectively decreased the affinity of classical H_2 agonists and antagonists but did not decrease the affinity of tricyclic antidepressants for H_2 receptors when evaluated indirectly by assessing the effectiveness of these agents on either stimulating or blocking stimulation of adenylate cyclase activity. The possibility that antidepressants and classical H_2 receptor antagonists may react with different histamine receptors seems unlikely because antidepressants were shown in the present study to be competitive antagonists of H_2 receptors as assessed by their ability to block activation of adenylate cyclase by either histamine or dimaprit, a specific H_2 receptor agonist.

Although the molecular basis underlying the selective effect of freezing and thawing is unknown, the evidence indicates that the interaction of histamine-sensitive adenylate cyclase with tricyclic antidepressants and H_2 receptor antagonists is somewhat different at the receptor level.

Investigators in prior reports have used gastric mucosal membranes which had been frozen to study histamine-sensitive adenylate cyclase [5, 30, 31]. Due to a marked decrease in the affinity of H_2 receptors for H_2 agonists and antagonists in the frozen–thawed membranes, the potency of these agents reported in earlier studies may have been considerably underestimated.

In light of the marked differences in the interaction of histamine H_2 receptor antagonists and tricyclic antidepressants with adenylate cyclase from guinea pig gastric mucosa found in our study, and in view of the discrepancy between the action of tricyclic antidepressants seen on H_2 receptors between pharmacological assays and that observed in biochemical assays, further studies are needed to understand the interaction of these agents with H_2 receptors.

Acknowledgements—The authors are grateful to Dr. Lee Limbird for her careful reading of this manuscript and suggestions. The authors also wish to express their appreciation to Ms. C. Ryan and Ms. D. Campbell for their assistance in the preparation of this manuscript.

REFERENCES

1. J. W. Black, in *Histamine Receptors* (Ed. T. O. Yellin), p. 23. Spectrum Press, New York (1979).
2. A. H. Soll and M. I. Grossman, *A. Rev. Med.* **29**, 495 (1978).
3. J. W. Black, W. A. M. Duncan, C. J. Furant, C. R. Ganellin and E. M. Parsons, *Nature, Lond.* **236**, 385 (1972).
4. G. C. Rosenfeld, S. J. Strada, E. J. Dial, C. J. Bearer

and W. J. Thompson, in *Advance in Cyclic Nucleotide Research* (Eds. P. Hamet and H. Sands), Vol. 12, p. 255. Raven Press, New York (1980).
5. T. P. Dousa and C. F. Code, *J. clin. Invest.* **53**, 334 (1974).
6. C. V. Perrier and M. Griessen, *Eur. J. clin. Invest.* **6**, 113 (1976).
7. S. Batzri and J. D. Gardner, *Molec. Pharmac.* **16**, 406 (1979).
8. A. H. Soll and A. Wollin, *Am. J. Physiol.* **237**, E444 (1979).
9. A. Wollin, A. H. Soll and I. M. Samloff, *Am. J. Physiol.* **237**, E437 (1979).
10. P. R. Bieck, in *International Conference on Stimulus-Secretion Coupling* (Eds. R. H. Case and H. Goebell), p. 129. Medical and Technical Publishing Co., Lancaster (1979).
11. F. G. Moody, *Am. J. Surg.* **135**, 731 (1978).
12. W. Domschke and S. Domschke, *Hepato-Gastroenterology* **27**, 163 (1980).
13. S. Bonfils, M. Dubrasquet and A. Lambling, *J. appl. Physiol.* **17**, 299 (1962).
14. W. Lippman and K. Seethaler, *Life Sci.* **20**, 1393 (1977).
15. S. Garattini, A. Giachetti, A. Jori, L. Pieri and L. Valzelli, *J. Pharm. Pharmac.* **14**, 509 (1962).
16. K. Valnes, J. Myren and T. Qvigstad, *Scand. J. Gastroent.* **13**, 497 (1978).
17. L. E. Borella and W. Lippman, *Eur. J. Pharmac.* **52**, 153 (1978).
18. T. Bohamn, J. Myren, O. Flaten and E. Schrumpf, *Scand. J. Gastroent.* **15**, 177 (1980).
19. J. P. Green and S. Marayani, *Nature, Lond.* **269**, 163 (1977).
20. P. D. Kanof and P. Greengard, *Nature, Lond.* **272**, 329 (1978).
21. B. S. Tsai and T. O. Yellin, *Life Sci.* **33**, 895 (1983).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
23. R. F. Furchgott, *Ann. N.Y. Acad. Sci.* **139**, 553 (1967).
24. T. O. Yellin, S. H. Buck, D. J. Gilman, D. F. Jones and J. M. Wardleworth, *Life Sci.* **25**, 2001 (1979).
25. A. M. Cheret, F. Pignal and J. J-M. Lewin, *Molec. Pharmac.* **20**, 326 (1982).
26. M. E. Parsons, D. A. A. Owen, C. R. Ganellin and G. J. Durant, *Agents Actions* **7**, 31 (1977).
27. H. O. Schild, *Pharmac. Rev.* **9**, 242 (1957).
28. J. A. Angus and J. W. Black, *Circulation Res.* **46** (Suppl. 1), 164 (1980).
29. M. D. T. Tuong, M. Garburg and J. C. Schwartz, *Nature, Lond.* **287**, 548 (1980).
30. A. Wollin, C. F. Code and T. P. Dousa, *J. clin. Invest.* **57**, 1548 (1976).
31. P. Anttila, C. Lucke and E. Westermann, *Naunyn-Schmiedeberg's Archs Pharmac.* **296**, 31 (1976).