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EFFECT OF A NEW POTENT H_2-RECEPTOR ANTAGONIST 3[[[2-[(DIAMINOMETHYLENE)AMINO]-4-THIAZOLYL]METHYL]THIO]-N^2-SULFAMOYLPROPIONAMIDINE (YM-11170) ON GASTRIC MUCOSAL HISTAMINE-SENSITIVE ADENYLATE CYCLASE FROM GUINEA PIG

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Abstract—The effect of 3[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N^2-sulfamoylpropionamidine (YM-11170), a new thiazole H_2-receptor antagonist bearing propionamidine at the terminus of a side chain, on histamine-sensitive adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); EC 4.6.1.1] of gastric mucosa from the guinea pig was studied and compared with that of cimetidine. YM-11170 displaced the concentration–stimulation curve of histamine-sensitive adenylate cyclase to the right with a pA_2 of 7.65 ($K_i = 2.25 \times 10^{-8}$ M). Stimulation of gastric adenylate cyclase by 0.1 mM histamine was competitively inhibited by YM-11170 and cimetidine in a dose-dependent manner, with IC_{50} values of 5.9×10^{-7} M and 1.4×10^{-5} M respectively. Hippocampal histamine-sensitive adenylate cyclase in the presence of 0.1 mM histamine was also competitively inhibited by YM-11170 with an IC_{50} of 1.1×10^{-7} M. YM-11170 did not affect Gpp(NH)p-, NaF-, PGE_2-stimulated or basal activity of the gastric adenylate cyclase. These data, together with other results, indicate that YM-11170 is a highly selective and potent H_2-receptor antagonist which competes with histamine at the receptor site on the histamine-sensitive adenylate cyclase.

In gastric mucosa from guinea pig, human, dog, rabbit and rat [1–5], the stimulation by histamine of adenylate cyclase or of accumulation of adenosine-3′,5′-monophosphate (cAMP) in the tissue is specifically blocked by H_2-receptor antagonists that block histamine-stimulated gastric acid secretion. It is well established that the stimulatory effect of histamine on HCl secretion from the gastric parietal cells is mediated through cAMP as a second messenger in various animal species including humans [6, 7]. During the course of screening H_2-receptor antagonists with an amidine structure at the side chain of thiazole, 3[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N^2-sulfamoylpropionamidine (YM-11170†; Fig. 1) was found to be a very potent H_2-receptor antagonist, with far greater antisecretory potency than that of cimetidine [8]; the pharmacological characteristics have been documented in detail [9–12]. The present paper describes mainly the potent inhibitory effect of YM-11170 on histamine-sensitive adenylate cyclase from guinea pig gastric mucosa in comparison with that of cimetidine.

MATERIALS AND METHODS

Chemicals. Prostaglandin E_2 (PGE_2)‡, carbachol, pyrilamine maleate, l-alprenolol *d*-tartrate, and ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid (EGTA) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A., and 3-isobutyl-1-methylxanthine (IBMX) was from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. 5′-GTP and other 5′-nucleotides were obtained from Boehringer-Mannheim GmbH, Mannheim, West Germany. The following compounds were commercially obtained: histamine · 2HCl (Nakarai Chemicals, Ltd., Kyoto, Japan); atropine · H_2SO_4 and serotonin creatinine sulfate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan); tetragastrin (San-a Pharmaceutical Co., Ltd., Tokyo, Japan); pentagastrin (ICI Pharmaceutical Co., Ltd., Osaka, Japan); [8-^{3}H]cAMP (27.5 Ci/mmole) and [^{3}H]prazosin (20.2 Ci/mmole; Radiochemical Centre, Amersham, England); and [4-^{3}H]clonidine · HCl (23.8 Ci/mmole), l-[propyl-1,2,3-^{3}H]dihydroalprenolol · HCl (48.6 Ci/mmole), 5-[1,2-^{3}H(N)]hydroxytryptamine creatinine sulfate (26.4 Ci/mmole) and l-[benzilic-4,4′-^{3}H(N)]QNB (quinuclidinyl benzilate, 33.1 Ci/mmole; New England Nuclear Corp., Boston, MA, U.S.A.). Metiamide was provided by Smith Kline & French Laboratories Ltd., Welwyn Garden City, England; prazosin by Chas. Pfizer & Co., Inc., New York, NY, U.S.A.; and cyproheptadine · HCl by Shohwa Shinyaku Co., Ltd., Nagoya, Japan. YM-11170, cimetidine and clonidine were given to us by

* Author to whom all correspondence should be sent.
† Famotidine, INN.
‡ Abbreviations: PGE_2, prostaglandin E_2; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid; IBMX, 3-isobutyl-1-methylxanthine; Gpp(NH)p, 5′-guanylylimidodiphosphate; and QNB, quinuclidinyl benzilate.

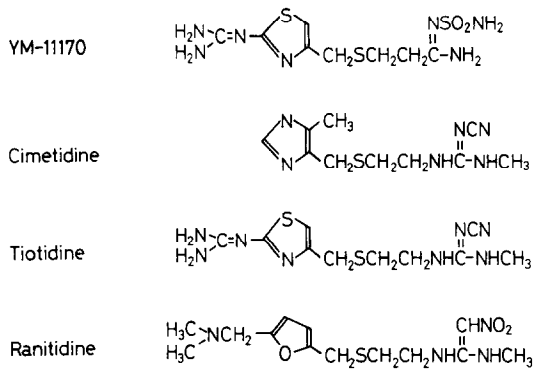

Fig. 1. Structures of four histamine H_2-receptor antagonists.

Dr. Y. Hirata and Dr. K. Niigata of our laboratories.

Preparation of membrane fragments from gastric mucosa. Fundic gastric mucosa was obtained from male Hartley guinea pigs, weighing 400–600 g, that had been fasted overnight according to the method of Wollin *et al.* [13]. The mucosa was gently homogenized in 6 vol. of ice-cold 10 mM Tris-maleate, pH 7.8, containing 3 mM $MgCl_2$, 2 mM EGTA and 0.25 M sucrose with a glass–Teflon homogenizer with 5 strokes. After centrifugation at 2000 *g* for 10 min, the sediment was resuspended in the ice-cold buffer without sucrose, and recentrifuged as described by Dousa and Code [1]. A suspension of the resulting pellets in the sucrose-free buffer was quickly frozen in dry ice and stored at −80° until it was used as the source of adenylate cyclase [3].

Preparation of hippocampal homogenates. The hippocampi dissected from male Hartley guinea pigs were homogenized in 100 vol. of ice-cold 10 mM Tris-maleate, pH 7.8, containing 2 mM EGTA in a glass homogenizer with a Teflon pestle. This homogenate was used as the source of adenylate cyclase within 30 min after homogenization [14].

Determination of adenylate cyclase activity. The adenylate cyclase activity was assayed according to the method of Kanof and Greengard [14], with a slight modification. The standard incubation mixture with a final volume of 0.5 ml contained 100 mM Tris-maleate, pH 7.8, 0.8 mM EGTA, 1 mM IBMX, 2 mM $MgCl_2$, 1 mM ATP, 0.01 mM GTP and an appropriate amount of enzyme with or without 0.1 mM histamine. Following preincubation of the enzyme in an ice-water bath for 10 min, the reaction was initiated by the addition of ATP, carried out for 10 min in a shaking water bath at 30°, and terminated by placing the assay tubes in a boiling water bath for 3 min. After centrifugation, aliquots (100 μl) of each supernatant fraction were assayed for cAMP by the protein binding method as described by Brown *et al.* [15]. The IC_{50} values (the concentration of each drug required for a half-maximal inhibition of histamine-sensitive adenylate cyclase) and the 95% confidence limits were calculated according to the simultaneous curve fitting of the data with a four-parameter logistic model [16].

Binding studies. The binding studies of [^{3}H]prazosin (0.4 nM) and [^{3}H]QNB (0.06 nM) were carried out according to the methods of Greengrass and Bremner [17] and Yamamura and Snyder [18], respectively, using membrane preparations from rat brain minus cerebellum and medulla. The binding of [^{3}H]clonidine (1.0 nM), [^{3}H]dihydroalprenolol (1.0 nM) and [^{3}H]serotonin (1.5 nM) was performed according to the methods of U'Prichard *et al.* [19], Bylund and Snyder [20] and Peroutka and Snyder [21], respectively, using rat cerebral cortex membranes. The specific binding of [^{3}H]prazosin, [^{3}H]clonidine, [^{3}H]dihydroalprenolol, [^{3}H]serotonin and [^{3}H]QNB was the difference between total binding and nonspecific binding in the presence of 6 μm prazosin, 3 μM clonidine, 10 μM l-alprenolol, 10 μM serotonin and 1 μM atropine respectively.

Protein determination. Protein was determined by the method of Lowry *et al.* [22] using bovine serum albumin as a standard.

RESULTS

General properties of gastric adenylate cyclase. About 80% of the total activity of histamine-sensitive adenylate cyclase in the homogenate of gastric mucosa was found in the 2000 *g* sediment, which was accordingly utilized as the enzyme source throughout the experiments. Under the standard conditions, the amount of cAMP formed increased almost linearly at least for the first 20 min with more than 5-fold stimulation by histamine.

As shown in Fig. 2 (left panel), histamine stimulated the adenylate cyclase in a dose-dependent manner with an apparent K_a (the concentration required for half-maximal stimulation) of 6.0 μM and a V_{max} (maximal reaction rate) of 24.5 pmoles per mg protein per min in the presence of a maximally effective concentration of GTP (10 μM), as computed according to the Lineweaver–Burk plot [23]. The maximally histamine-stimulated activity was more than five times as great as the basal activity. Considerable stimulation of the cyclase by histamine was also observed in the absence of added GTP, with a K_a of 4.3 μM and a V_{max} of 14.0 pmoles per mg protein per min (Fig. 2, left panel). Other secretagogues such as carbachol, tetragastrin and pentagastrin had no effect on the basal and histamine-sensitive adenylate cyclase regardless of the presence of 10 μM GTP, in agreement with the result reported by Anttila *et al.* [24]. On the other hand, PGE_2 exhibited dose-dependent stimulation of the adenylate cyclase with an apparent K_a of 0.26 μM, but to a lesser degree than did histamine, and GTP was virtually ineffective on PGE_2-stimulated activity (Fig. 2, right panel).

Effects of YM-11170 on gastric adenylate cyclase. Histamine-sensitive adenylate cyclase was determined in the presence of concentrations of YM-11170 between 10^{-8} M and 10^{-6} M. As shown in Fig. 3, increasingly higher concentrations of YM-11170 caused increasing shifts of the histamine dose-dependent curves to the right, indicating simple competitive antagonism. Similar experiments were performed with cimetidine between 3×10^{-7} M and 3×10^{-5} M and compared with those for YM-11170 according to Arunlakshana and Schild [25], as shown in Fig. 4. The straight lines for both drugs are parallel, and the slopes were estimated from the regression analysis to be 1.09 and 0.96 for YM-11170 and

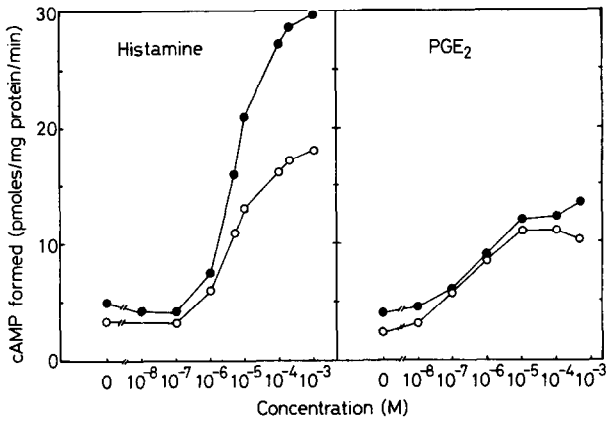

Fig. 2. Effects of various concentrations of histamine and PGE_2 on guinea pig gastric mucosal adenylate cyclase in the absence (○) or presence (●) of 10^{-5} M GTP. The reaction was carried out under the standard conditions except for the presence of the indicated concentrations of histamine (left panel) or PGE_2 (right panel). The enzyme amount was 60 μg protein. The results represent the means of duplicate incubations.

cimetidine, respectively, revealing the competitive nature of antagonism by these two compounds. The pA_2 values were estimated from the intercepts at the zero ordinate to be 7.65 for YM-11170 and 6.33 for cimetidine. From these pA_2 values were derived apparent K_i values of 2.25×10^{-8} M and 4.65×10^{-7} M for YM-11170 and cimetidine, respectively, indicating that YM-11170 was twenty times more potent than cimetidine ($P < 0.01$).

At a fixed concentration of histamine (0.1 mM), stimulation of adenylate cyclase was progressively reduced by increasing concentrations of YM-11170 and cimetidine (Fig. 5). Representation of the data according to simultaneous curve fitting with the four-parameter logistic model [16] resulted in two parallel lines with slopes not different from unity (1.01 for YM-11170 and 0.97 for cimetidine). This finding again argues strongly that YM-11170 is a purely competitive H_2-receptor antagonist. The IC_{50}

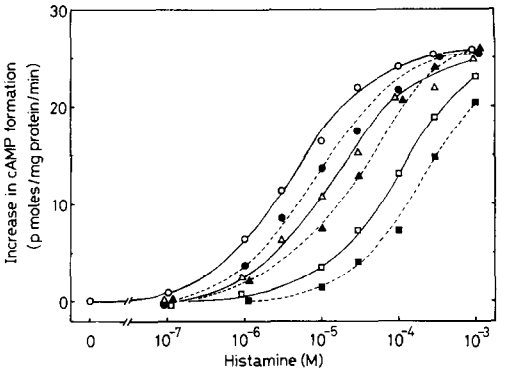

Fig. 3. Effects of YM-11170 on the stimulation of guinea pig gastric mucosal adenylate cyclase by various concentrations of histamine. The reaction was carried out as described in the legend to Fig. 2 in the presence of 10^{-5} M GTP. Basal adenylate cyclase activity was 3.72 pmoles per mg protein per min. YM-11170 alone did not affect the basal activity. The results represent the means of duplicate incubations. Key: control (—○—); YM-11170 at 10^{-8} M (—●—), 3×10^{-8} M (—△—), 10^{-7} M (—▲—), 3×10^{-7} M (—□—), and 10^{-6} M (—■—).

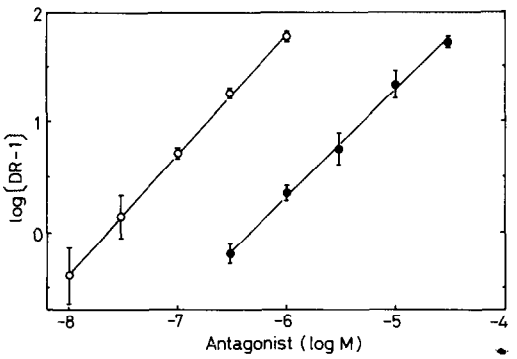

Fig. 4. Schild plots of the inhibition by YM-11170 (○) and cimetidine (●) of histamine-sensitive adenylate cyclase in guinea pig gastric mucosa. The dose ratios (DR) were calculated from the ratio of the ED_{50} values for histamine in the presence versus the absence of various concentration of the H_2-receptor antagonists. Each point is the mean ± S.E. of three and two separate experiments for YM-11170 and cimetidine respectively.

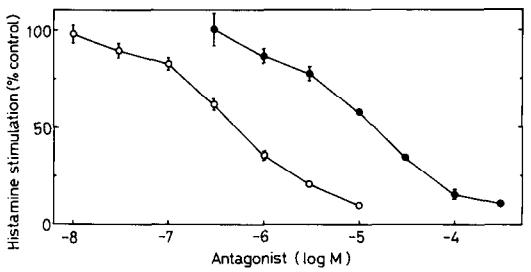

Fig. 5. Effects of various concentrations of YM-11170 (○) and cimetidine (●) on stimulation of guinea pig gastric mucosal adenylate cyclase by 0.1 mM histamine. Histamine-stimulated activities in the presence of various concentrations of H_2-receptor antagonists were determined under the standard conditions containing 60 μg protein and are expressed as percent of control. The 0.1 mM histamine-stimulated activity of adenylate cyclase in the absence of drugs was 30.8 ± 1.7 pmoles per mg protein per min (N = 13). Each value represents the mean ± S.E. of eight and five experiments for YM-11170 and cimetidine respectively. The results without S.E. indicate that S.E. was less than 1.7%.

Table 1. IC_{50} Values of histamine-sensitive adenylate cyclase from guinea pig gastric mucosa and hippocampus*

Drug	IC_{50} (M)	
	Gastric mucosa	Hippocampus
H_2-Antagonist		
YM-11170	5.9×10^{-7}†	1.1×10^{-7}
Cyproheptadine	2.6×10^{-7}	1.3×10^{-7}
Metiamide	1.3×10^{-5}	1.3×10^{-5}
Cimetidine	1.4×10^{-5}†	1.3×10^{-5}
H_1-Antagonist		
Pyrilamine	5.2×10^{-5}	5.7×10^{-5}
Antimuscarinic		
Atropine	1.6×10^{-3}	9.1×10^{-4}

* Histamine-sensitive adenylate cyclase was determined under the standard conditions in the presence of five to seven different concentrations of the drugs. Experiments were repeated three to four times for each drug.
† Data from Fig. 5.

values were 5.9×10^{-7} M and 1.4×10^{-5} M for YM-11170 and cimetidine, respectively, indicating that YM-11170 was twenty-four times more potent than cimetidine ($P < 0.001$). According to the simultaneous curve fitting of the dose–response curves, 95% confidence limits were estimated to be 5.0–6.9×10^{-7} M and 1.1–1.7×10^{-5} M for YM-11170 and cimetidine respectively. Assuming that the type of inhibition was competitive, K_i values were calculated to be 3.1×10^{-8} M and 7.2×10^{-7} M for YM-11170 and cimetidine, respectively, employing the IC_{50} values. YM-11170 did not alter the basal activity of adenylate cyclase nor the stimulated activity by 10^{-4} M PGE_2, 10^{-4} M Gpp(NH)p and 10^{-2} M NaF.

The effects of other H_2-receptor antagonists on gastric histamine-sensitive adenylate cyclase were also investigated in the presence of 0.1 mM histamine. Cyproheptadine and metiamide dose-dependently inhibited histamine-sensitive adenylate cyclase without affecting the basal activity. Table 1 summarizes the IC_{50} values for the H_2-receptor antagonists tested, in which YM-11170 was the most potent selective H_2-receptor antagonist.

Comparison with hippocampal histamine-sensitive adenylate cyclase. Under the standard assay conditions, histamine elevated hippocampal adenylate cyclase activity in a dose-dependent manner with a K_a of 9.1 μM and a V_{max} of 102 pmoles per mg protein per min (Fig. 6). In great contrast to gastric adenylate cyclase, histamine produced little stimulation of hippocampal enzyme in the absence of added GTP, in agreement with the report by Kanof *et al.* [26]. Because of this striking difference in the requirement for GTP between gastric and hippocampal histamine-sensitive adenylate cyclase, we examined the effects of H_2-receptor antagonists on the latter enzyme.

YM-11170 shifted a histamine concentration–stimulation curve of the adenylate cyclase to the right in an apparently competitive fashion as described by Kanof and Greengard [27] with cimetidine; the IC_{50} values, which were obtained from the dose–response curves, are shown in Table 1. Hippocampal histamine-sensitive adenylate cyclase was considerably inhibited by YM-11170 and cyproheptadine, while the basal activity was unaffected. Metiamide and cimetidine revealed a far less inhibitory effect than YM-11170. Pyrilamine and atropine were rather weak inhibitors for the hippocampal and gastric cyclases. Thus, there was no substantial difference between the inhibitory effects of the drugs on the gastric and hippocampal histamine-sensitive adenylate cyclases.

Effects of YM-11170 on the specific binding of ^{3}H-labeled ligands to the rat brain receptors. Table 2 shows the affinity of YM-11170 for adrenergic (α_1, α_2 and β), serotonergic (5-HT_1) and cholinergic (muscarinic) receptors. YM-11170 was substantially inactive in these binding tests. Cimetidine also exhibited very weak affinity for the receptors.

DISCUSSION

YM-11170 is a structurally new type of H_2-receptor antagonist with propionamidine, instead of ethylguanidine (cimetidine and tiotidine) or ethyl-

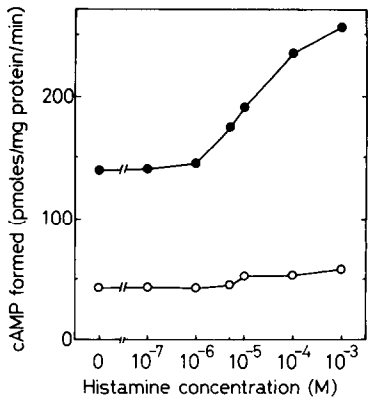

Fig. 6. Effects of various concentrations of histamine on guinea pig hippocampal adenylate cyclase activity in the absence (○) or presence (●) of 10^{-5} M GTP. The reaction was carried out as described in the legend to Fig. 2 except for the enzyme amount (54 μg protein). The results represent the means of duplicate incubations.

Table 2. Effects of YM-11170 and cimetidine on the specific binding of ^{3}H-labeled ligands to rat brain receptors*

Ligand	Specific binding (% of control)	
	10^{-5} M YM-11170	10^{-5} M cimetidine
[^{3}H]Prazosin	91.3 ± 1.2 (8)	93.1 ± 1.6 (6)
[^{3}H]Clonidine	98.6 ± 7.6 (9)	88.9 ± 3.5 (6)
[^{3}H]Dihydroalprenolol	106.9 ± 2.9 (6)	99.8 ± 1.2 (5)
[^{3}H]Serotonin	74.8 ± 3.1 (6)	99.3 ± 3.9 (6)
[^{3}H]QNB	96.0 ± 1.2 (9)	91.9 ± 1.7 (6)

* Binding was carried out under the conditions described in Materials and Methods using [^{3}H]prazosin [17], [^{3}H]clonidine [19], [^{3}H]dihydroalprenolol [20], [^{3}H]serotonin [21] and [^{3}H]QNB [18]. The values are means ± S.E. of incubations whose number is shown in parentheses. The specific binding in the absence of the drugs was: [^{3}H]prazosin, 83.6 ± 1.3 fmoles/mg protein (N = 19); [^{3}H]clonidine, 61.0 ± 3.1 fmoles/mg protein (N = 13); [^{3}H]dihydroalprenolol, 64.4 ± 1.6 fmoles/mg protein (N = 7); [^{3}H]serotonin, 56.7 ± 2.1 fmoles/mg protein (N = 20); and [^{3}H]QNB, 287.7 ± 16.8 fmoles/mg protein (N = 13).

ethenediamine (ranitidine) (Fig. 1), and reduces histamine-induced gastric acid secretion in a competitive manner [28]. The present experiments demonstrate that YM-11170 inhibits competitively histamine-sensitive adenylate cyclase in gastric mucosa of the guinea pig. The K_i (2.25×10^{-8} M) and pA_2 (7.65) values estimated from the displacement of concentration-stimulation curves according to the method of Arunlakshana and Schild [25] appear to be highly consistent with the IC_{50} (5.9×10^{-7} M) derived from inhibition curves at a fixed concentration of histamine (10^{-4} M).

YM-11170 was about twenty-four times as potent as cimetidine in the inhibition of histamine-sensitive adenylate cyclase. Since the pA_2 values for tiotidine and ranitidine are reported to be 7.62 and 6.90 [29], respectively, YM-11170 appears to be almost equipotent to tiotidine, the most potent H_2-receptor antagonist to date. Based on these observations, all four competitive inhibitors seem to show the following order of potencies: YM-11170 = tiotidine > ranitidine > cimetidine. It has been generally thought that H_2-receptor antagonists require side chains that resemble the ethylamine side chain of histamine in order to interact with H_2-receptors, but with modifications resulting in increased chain length and deprotonation of the NH_2 terminus as seen with cimetidine, ranitidine and tiotidine (Fig. 1). However, such a view is no longer valid, since YM-11170 with a propionamidine group instead of the ethylamine structure in tiotidine is also a very potent H_2-receptor antagonist, being approximately equipotent to tiotidine.

YM-11170 has virtually no affinity for adrenergic (α_1, α_2 and β), serotonergic (5-HT_1) and muscarinic receptors as determined by the radioligand binding method, indicating it to be a rather specific H_2-receptor antagonist. This high selectivity of YM-11170 for the H_2-receptor has been pharmacologically verified by Takeda *et al.* [9].

In view of the enzymatic characteristics of thoroughly washed membranes, it is of interest to note that gastric adenylate cyclase can be stimulated several-fold by histamine in the absence of added GTP as shown in previous [30] and present experiments, whereas hippocampal and cardiac histamine-sensitive adenylate cyclases absolutely require exogenous addition of GTP for its stimulation by histamine [31]. Particularly noteworthy in the present experiments with the gastric enzyme is that increasing concentrations of Gpp(NH)p between 10^{-6} M and 10^{-4} M increased the basal activity without altering significantly the histamine sensitivity. The Gpp(NH)p-induced activity, as well as the basal activity, is not reduced by YM-11170. Furthermore, the previous report indicates that Gpp(NH)p at less than 10^{-6} M considerably inhibits histamine-sensitive adenylate cyclase of gastric parietal cell membranes from the guinea pig [30]. These observations distinguish clearly this particular gastric histamine-sensitive adenylate cyclase from a variety of other hormone-sensitive adenylate cyclases including hippocampal and cardiac histamine-sensitive adenylate cyclases. The gastric adenylate cyclase was also stimulated by PGE_2 regardless of the presence of added GTP, but the stimulation was not influenced by YM-11170. The results indicate that YM-11170 acts on neither the guanine nucleotide regulatory protein nor the catalytic subunit, but specifically on the histamine binding site, thereby inhibiting the histamine-sensitive adenylate cyclase competitively with respect to histamine.

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REFERENCES

1. T. P. Dousa and C. F. Code, *J. clin. Invest.* **53**, 334 (1974).
2. B. Simon and H. Kather, *Gastroenterology* **73**, 429 (1977).
3. R. R. Dozois, A. Wollin, R. D. Rettmann and T. P. Dousa, *Am. J. Physiol.* **232**, E35 (1977).
4. C. P. Sung, B. C. Jenkins, L. R. Burns, V. Hackney, J. G. Spenney, G. Sachs and V. D. Wiebelhaus, *Am. J. Physiol.* **225**, 1359 (1973).

5. J. H. McNeill and S. C. Verma, *Br. J. Pharmac.* **52**, 104 (1974).
6. T. P. Dousa and R. R. Dozois, *Gastroenterology* **73**, 904 (1977).
7. A. H. Soll, R. Rodrigo and J. C. Ferrari, *Fedn Proc.* **40**, 2519 (1981).
8. I. Yanagisawa, Y. Hirata, Y. Ishii, M. Takeda and T. Takagi, in *Proceeding of the 101th Annual Meeting of the Pharmaceutical Society of Japan*, p. 353, Pharmaceutical Society of Japan, Tokyo (1981).
9. M. Takeda, T. Takagi and H. Maeno, *Jap. J. Pharmac.* (Suppl.) **31**, 222P (1981).
10. K. Tomioka and T. Yamada, *Archs int. Pharmacodyn. Thér.* **255**, 16 (1982).
11. T. Takagi, M. Takeda and H. Maeno, *Archs int. Pharmacodyn. Thér.* **256**, 49 (1982).
12. M. Takeda, T. Takagi, Y. Yashima and H. Maeno, *Arzneimittel-Forsch.* **32**, 734 (1982).
13. A. Wollin, C. F. Code and T. P. Dousa, *J. clin. Invest.* **57**, 1548 (1976).
14. P. D. Kanof and P. Greengard, *Nature, Lond.* **272**, 329 (1978).
15. B. L. Brown, R. P. Ekins and J. D. M. Albano, *Adv. Cyclic Nucleotide Res.* **2**, 25 (1972).
16. A. DeLean, P. J. Munson and D. Rodbard, *Am. J. Physiol.* **235**, E97 (1978).
17. R. Greengrass and R. Bremner, *Eur. J. Pharmac.* **55**, 323 (1979).
18. H. I. Yamamura and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **71**, 1725 (1974).
19. D. C. U'Prichard, D. A. Greenberg and S. H. Snyder, *Molec. Pharmac.* **13**, 454 (1977).
20. D. B. Bylund and S. H. Snyder, *Molec. Pharmac.* **12**, 568 (1976).
21. S. J. Peroutka and S. H. Snyder, *Molec. Pharmac.* **16**, 687 (1979).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
23. M. Dixon and E. C. Webb, in *Enzymes*, p. 60. Longmans Group Ltd., London (1979).
24. P. Anttila, C. Lücke and E. Westermann, *Naunyn-Schmiedeberg's Archs Pharmac.* **296**, 31 (1976).
25. O. Arunlakshana and H. O. Schild, *Br. J. Pharmac.* **14**, 48 (1959).
26. P. D. Kanof, L. R. Hegstrand and P. Greengard, *Archs Biochem. Biophys.* **182**, 321 (1977).
27. P. D. Kanof and P. Greengard, *J. Pharmac. exp. Ther.* **209**, 87 (1979).
28. M. Takeda, T. Takagi, Y. Yashima and H. Maeno, in *Proceedings of the Eighth International Congress of Pharmacology*, p. 692, Japanese Pharmacological Society & Science Council of Japan, Tokyo (1981).
29. A. M. Cheret, F. Pignal and J-M. Lewin, *Molec. Pharmac.* **20**, 326 (1981).
30. M. Harada and H. Maeno, *Archs Biochem. Biophys.* **213**, 405 (1982).
31. C. L. Johnson, H. Weinstein and J. P. Green, *Biochim. biophys. Acta* **587**, 155 (1979).