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# Pharmacological Characterization of Histamine Receptors Mediating Cyclic AMP Accumulation in the Mouse Vas Deferens

## Sensitivity to $H_1$ and $H_2$ Receptor Agonists and Antagonists

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SUMMARY

Histamine ($10^{-6}$–$10^{-2}$ M) caused a concentration-dependent increase in the accumulation of endogenous cyclic AMP in mouse vas deferens of about 3 times the basal level. This effect was mimicked by dimaprit and 4-methylhistamine, two known $H_2$ receptor agonists, and, to a lesser extent, by 2-thiazolylethylamine, a relatively selective $H_1$ receptor agonist. The slopes of the percentage of maximal increase in cyclic AMP accumulation versus log molar concentration curves for the $H_2$ agonists did not differ significantly from that for histamine. The rank order and relative potencies of the tested $H_2$ agonists were as follows: histamine (100%) > dimaprit (64%) > 4-methylhistamine (31%). The effect of $10^{-2}$ M (but not of $10^{-3}$ M) 2-thiazolylethylamine on cyclic AMP accumulation was markedly depressed in the presence of 1 μM propranolol. Cyclic AMP accumulation elicited by $10^{-3}$ M 2-thiazolylethylamine was significantly lower than that caused by histamine, indicating that the intrinsic activity of 2-thiazolylethylamine relative to histamine was considerably lower; therefore, its relative potency as an $H_2$ agonist could not be defined. Stimulation of cyclic AMP accumulation caused by histamine was antagonized by cimetidine (3.5–140 μM), an $H_2$ receptor antagonist, in a concentration-related manner, but not by mepyramine (0.1–1.0 μM), an $H_1$ receptor antagonist. The slope of the Schild plot for cimetidine against histamine-induced cyclic AMP accumulation was not significantly different from unity, confirming that cimetidine causes a competitive inhibition of histamine response. The calculated $pA_2$ value of cimetidine against histamine was 5.40. 2-Thiazolylethylamine-stimulated cyclic AMP accumulation was also antagonized by cimetidine (35 μM) but was unaffected by mepyramine (1.0 μM). On the basis of these findings, it is concluded that the stimulant effect of histamine and selective $H_1$ and $H_2$ receptor agonists on cyclic AMP accumulation in mouse vas deferens is mediated via activation of a histamine $H_2$ receptor in the preparation. Although the potency profile of the various $H_2$ agonists as determined in the present study is similar to that reported for typical $H_2$ receptor-containing systems such as guinea pig atrium, the calculated $pA_2$ value of cimetidine in mouse vas deferens is lower than that reported in guinea pig atrium. In this regard, 2-thiazolylethylamine seems to act as a partial agonist. Furthermore, the maximal increase in cyclic AMP accumulation elicited by 2-thiazolylethylamine, unlike that elicited by histamine, was significantly depressed by propranolol (1.0 μM), indicating that the effect of 2-thiazolylethylamine at the highest concentration on cyclic AMP accumulation might in part be secondary to the release of norepinephrine from the endogenous stores.

## INTRODUCTION

The concept of two distinct types of pharmacological receptors ($H_1$ and $H_2$) for histamine actions is now well established (1–3). The histamine actions produced by $H_1$ receptors (such as bronchoconstriction, vasoconstriction, stimulation of ileal preparations, and partially increased capillary permeability) are blocked by classical antihistamines [such as mepyramine (pyrilamine)]; those mediated by $H_2$ receptors (such as cardiostimulation, increased gastric acid secretion, and increased cyclic AMP formation) are blocked by metiamide or cimetidine. Relatively selective $H_1$ and $H_2$ receptor agonists have recently been synthesized and have become available (4, 5). These agonists, in conjunction with corresponding antagonists, make it possible to identify and characterize receptors mediating the actions of histamine in various

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tissues and organs. Investigations using these compounds have thus far revealed that the distribution of $H_1$ and $H_2$ receptors varies among animal species and among the types of tissue under study (6).

In several species, the male accessory genital organs (such as prostate, seminal vesicle, and vas deferens) contain relatively high concentrations of histamine (7, 8). Although several recent reports have described the actions of histamine and the characterization of its receptors in the seminal vesicle and vas deferens of different species (9–13), the actions of histamine and the nature of its receptors in these organs are still largely unclear. Recent studies in this laboratory (9, 10) revealed that mouse vas deferens contains an $H_2$ receptor but apparently no $H_1$ receptor. We found that, via a cimetidine-sensitive ($H_2$) receptor, histamine markedly inhibits contractions of mouse vas deferens stimulated by electrical field stimulation or by exogenous norepinephrine.

There is substantial evidence that a metiamide- or cimetidine-sensitive ($H_2$) receptor may be coupled to an adenylate cyclase-cyclic AMP system in heart (14, 15), gastric mucosa (16), brain (17), and other tissues and organs. The present study was therefore designed to investigate the effect of histamine on the accumulation of endogenous cyclic AMP in mouse vas deferens and to characterize the receptor(s) mediating the stimulation of cyclic AMP accumulation in this tissue, using selective $H_1$ and $H_2$ receptor agonists and antagonists. Histamine is a mixed agonist, as it stimulates both $H_1$ and $H_2$ receptors. Dimaprit is a selective $H_2$ receptor agonist, and 4-methylhistamine is a relatively selective $H_2$ receptor agonist; 2-thiazolylethylamine is a relatively selective $H_1$ receptor agonist.

## MATERIALS AND METHODS

*Animals.* Randomly bred male Swiss-Webster mice of the HPB strain weighing 25–30 g were obtained from the High Oak Ranch (Goodwood, Ont., Canada).

*Preparation of vasa deferentia.* Mice were killed by cervical dislocation. The abdomen was opened by a midline incision; both vasa deferentia were exposed, dissected out, and placed in oxygenated Krebs-Ringer bicarbonate solution containing (millimolar concentrations) NaCl, 115.5; KCl, 4.63; $CaCl_2$, 2.47; $MgCl_2$, 1.16; $NaH_2PO_4$, 1.16; $NaHCO_3$, 21.9; and glucose, 11 (pH 7.4). There they were carefully stripped of adhering fat, connective tissue, and main blood vessels, as described previously (9). Unless stated otherwise, each stripped vas deferens was transversely sectioned into two pieces. Each piece represented a tissue sample and was assigned to a separate treatment.

These tissues were equilibrated for at least 1 hr in Krebs solution maintained at 36° and continuously gassed with 95% $O_2$/5% $CO_2$. Thereafter, they were transferred into Krebs solution containing 5 mM aminophylline, with and without various concentrations of selective antagonist, and allowed to equilibrate for another 30 min. Various concentrations of histamine and selective $H_1$ and $H_2$ receptor agonists were then added to the equilibration solution, and the tissues were incubated for 5 min. The incubations were terminated by adding ice-cold trichloroacetic acid solution to a final concentration of 10% (w/v), each sample was spiked with cyclic [$^3$H]AMP (10 μl containing approximately 1000 cpm) as internal marker for the recoveries of cyclic AMP, and the tissues were homogenized immediately in a glass homogenizer fitted with a Teflon pestle.

*Determination of cyclic AMP content.* Homogenates were centrifuged at 3000 × *g* and 4° for 30 min in a Sorvall RB-2 refrigerated centrifuge. The supernatant was removed and purified by Dowex-50 column chromatography (AG 50W-X8, 100–200 mesh, hydrogen form; obtained from Bio-Rad Laboratories, Richmond, Calif.) according to the method of Krishna and Birnbaumer (18). Fractions containing cyclic AMP eluted in 3–6 ml of distilled water were combined and lyophilized to dryness. The residue was reconstituted in 0.4 ml of 0.05 M sodium acetate buffer (pH 6.2). Duplicate samples of 10, 20, and 40 μl were used to determine the cyclic AMP content by radioimmunoassay using a cyclic AMP $^{125}$I radioimmunoassay kit (New England Nuclear Corporation, Boston, Mass.). Duplicate samples of 100 μl of the reconstituted sample were also added to scintillation vials containing 10 ml of Bio-Fluor, and total radioactivity was determined on a liquid scintillation spectrometer (Mark II, Nuclear Chicago, Chicago, Ill.) using the dual channel ratio counting procedure. Recoveries of cyclic [$^3$H]AMP ranged from 70% to 85%. The results were corrected for the appropriate losses of cyclic AMP. Proteins were determined by Hartree's modification (19) of the method of Lowry *et al.* The data are expressed as picomoles of cyclic AMP formed during the incubation period per milligram of protein. The results are presented as means ± standard error of the mean.

*Analysis of data.* To establish net accumulation of cyclic AMP at various concentrations of each agonist, the mean basal cyclic AMP values were subtracted from the mean stimulated cyclic AMP accumulation values. These data were then expressed as percentages of maximal stimulation elicited by each compound. Regression lines were fitted to the linear portion of the concentration-response curves by the method of least squares. From these curves, the effective concentration of each agonist required to produce 50% of its maximal effect ($EC_{50}$) was determined to establish the equipotent molar ratio for the calculation of relative potency. The relative potency of an agonist was calculated by using the formula: relative potency = ($EC_{50}$ histamine/$EC_{50}$ agonist) × 100.

Dose ratios were calculated from the $EC_{50}$ of histamine determined in the absence and presence of different concentrations of cimetidine. The −log of the molar concentration ($pA_2$) was determined from Arunlakshana-Schild plots (20), using cimetidine (3.5–140 μM) and incubating the tissues for at least 30 min before the concentration-response curve for histamine was determined. Confidence limits for the slope of the Schild plot were determined as described by Goldstein (21).

*Drugs and chemicals.* Histamine dihydrochloride, (±)-propranolol HCl, and aminophylline were obtained from Sigma Chemical Company (St. Louis, Mo.); mepyramine maleate was obtained from Poulenc Ltd. (Montreal, Canada). Cimetidine HCl, 2-thiazolylethylamine dihydrochloride, dimaprit dihydrochloride, and 4-methylhistamine dihydrochloride were generously provided by Dr. C. R. Ganellin (The Research Institute,

Smith Kline & French Laboratories, Hertfordshire, England). The cyclic AMP $^{125}I$ radioimmunoassay kit was obtained from New England Nuclear Corporation.

## RESULTS

*Effect of aminophylline on basal and on histamine-stimulated cyclic AMP levels.* The basal cyclic AMP levels in mouse vas deferens untreated with aminophylline were 11.9 ± 2.6 pmoles/mg of protein. When the tissues were incubated with 5 mM aminophylline, their basal cyclic AMP levels increased with time, reaching a maximum of 30.3 ± 3.7 pmoles/mg of protein at 30 min and remaining reasonably steady for 30 min thereafter (Fig. 1A). The maximal increase in the basal cyclic AMP level was about 3 times greater when the equilibration solution contained aminophylline than when it did not.

Cyclic AMP levels increased in tissues exposed to 100 μM histamine, whether aminophylline was present or absent; however, the increase was considerably greater in tissues treated with aminophylline. The increase in cyclic AMP accumulation above the basal level was only 1.5-fold in the absence of aminophylline, compared to a 3-fold increase in those tissues that had been equilibrated with aminophylline for 30 min (Fig. 1B). Whether or not the tissues had been exposed to aminophylline, the maximal histamine-stimulated increase in their cyclic AMP levels occurred about 5 min after the exposure to histamine and remained steady at about this level for up to 30 min of exposure to the drug.

On the basis of these findings, we adopted a 30-min equilibration period in Krebs solution containing 5 mM aminophylline followed by a 5-min exposure to histamine or other agonists in all subsequent studies.

*Effect of histamine and selective $H_1$ and $H_2$ receptor agonists.* Histamine in concentrations ranging from $10^{-6}$ to $10^{-2}$ M increased cyclic AMP accumulation in mouse vas deferens to about 3 times the mean basal level in a dose-dependent manner. The threshold concentration of histamine required to produce an increase in the cyclic AMP level was 1 μM, and the maximal elevation occurred at 1 mM (Fig. 2). Two selective $H_2$ receptor agonists (dimaprit and 4-methylhistamine) and a selective $H_1$ receptor agonist (2-thiazolylethylamine) produced stimulatory effects on cyclic AMP accumulation similar to that produced by histamine. The calculated relative potencies and the rank order of the $H_2$ receptor agonists were as follows: histamine (100%) > dimaprit (64%) > 4-methylhistamine (31%). Although the selective agonists were less potent than histamine, the differences between their maximal stimulations of cyclic AMP accumulation and the slopes of their concentration-effect curves, and those of histamine, were not statistically significant ($p > 0.05$).

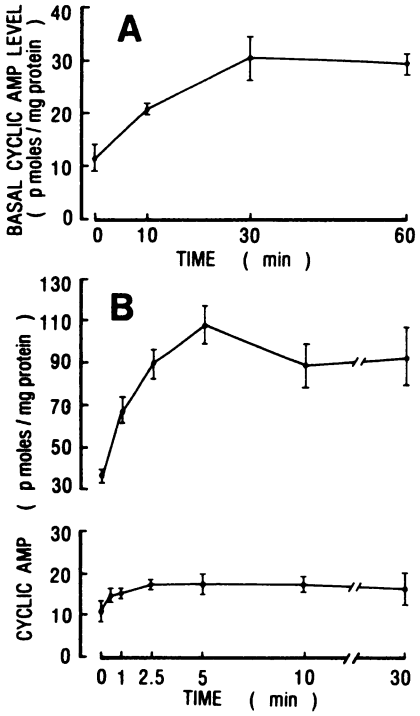


FIG. 1. *Effect of 5 mM aminophylline on basal cyclic AMP levels (A) and of 100 μM histamine on cyclic AMP accumulation in the presence (top) and absence (bottom) of 5 mM aminophylline (B) in mouse vas deferens*

A. Tissues equilibrated for at least 1 hr in normal Krebs solution were transferred to Krebs solution containing 5 mM aminophylline and assayed for cyclic AMP at different times. Each value represents the mean ± standard error ($n = 3$).

B. Tissues equilibrated for at least 1 hr in normal Krebs solution were transferred to either 5 mM aminophylline-Krebs solution (*top*) or normal Krebs solution (*bottom*) and equilibrated for another 30 min. Histamine (100 μM) was then added and the tissues were assayed for cyclic AMP at different times. Values are means ± standard error ($n = 4$–6). Whole vas deferens was used in the case of tissues equilibrated in normal Krebs solution.

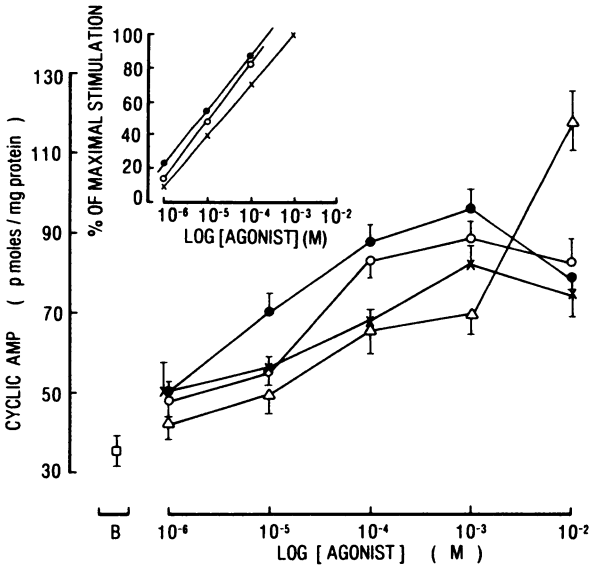


FIG. 2. *Concentration-response curve for cyclic AMP accumulation caused by histamine and $H_1$ and $H_2$ receptor agonists in mouse vas deferens*

After incubation for 30 min in 5 mM aminophylline-Krebs solution, tissues were exposed for 5 min to histamine (●), dimaprit (○), 4-methylhistamine (×), and 2-thiazolylethylamine (△) and assayed for cyclic AMP content. The control mean basal cyclic AMP level is shown at *B* (□). Each value represents the mean ± standard error of 6–12 tissues. The *inset* shows the fitted regression lines calculated from the results, expressed as percentage of maximal stimulation caused by each agonist.

On the other hand, 2-thiazolylethylamine caused a maximal stimulation of cyclic AMP accumulation at $10^{-2}$ M. However, as we describe later, the accumulation of cyclic AMP caused by 2-thiazolylethylamine at $10^{-2}$ M but not at $10^{-3}$ M was secondary in part to the release of norepinephrine, since it was significantly depressed in the presence of propranolol (1 μM). Therefore, in the determination of its potency relative to that of histamine, the accumulation of cyclic AMP produced by 2-thiazolylethylamine at $10^{-3}$ M was compared with that of histamine. This accumulation of cyclic AMP caused by $10^{-3}$ M 2-thiazolylethylamine was significantly lower ($p < 0.05$) than that caused by histamine. This indicates that 2-thiazolylethylamine has an intrinsic activity lower than that of histamine in stimulating cyclic AMP accumulation in mouse vas deferens, and thus it seems to act as a partial agonist in this regard. Consequently, its relative potency could not be defined, as it failed to meet the important criterion (i.e., its maximal effect was not the same as that produced by histamine).

*Effect of mepyramine, cimetidine, and propranolol on histamine-stimulated cyclic AMP accumulation.* The concentration-effect curves in Fig. 3 show that the histamine stimulation of cyclic AMP accumulation was apparently unaffected in the presence of 0.1 μM mepyramine, an $H_1$ receptor antagonist, but was markedly enhanced in the presence of 1 μM mepyramine. In contrast, the concentration-effect curve for histamine (Fig. 4) was progressively displaced to the right with increasing concentrations of cimetidine, an $H_2$ receptor antagonist,

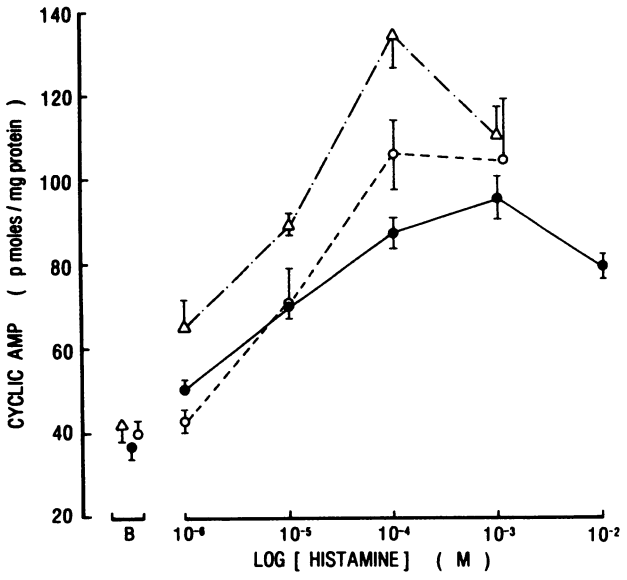


FIG. 3. *Effect of mepyramine on histamine-stimulated cyclic AMP accumulation in mouse vas deferens*

Tissues equilibrated for 1 hr in normal Krebs solution were incubated for 30 min in 5 mM aminophylline-Krebs solution containing an appropriate concentration of mepyramine; histamine then was added. The curves show cyclic AMP accumulation after a 5-min incubation with histamine alone (●), $n = 6$–12; histamine plus 0.1 μM mepyramine (○), $n = 6$; and histamine plus 1.0 μM mepyramine (△), $n = 3$. Each value represents the mean ± standard error. Basal levels of cyclic AMP (shown at *B*) were not significantly altered by the different concentrations of mepyramine.

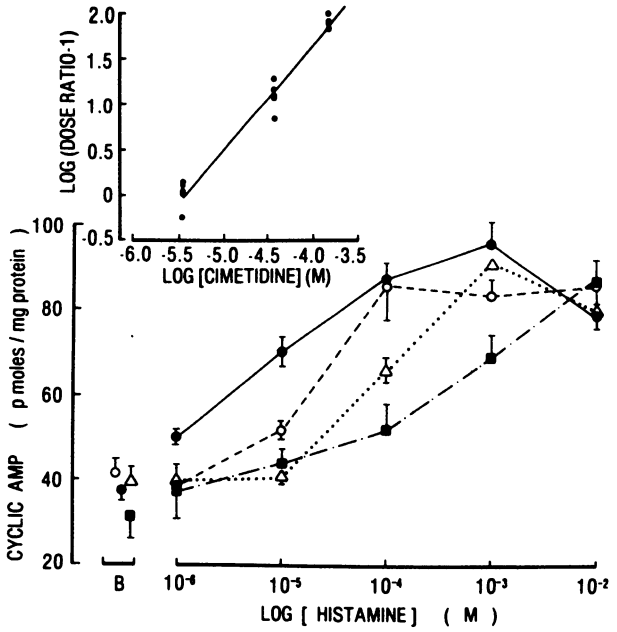


FIG. 4. *Effect of cimetidine on histamine-induced cyclic AMP accumulation in mouse vas deferens*

Tissues equilibrated for 1 hr in normal Krebs solution were incubated for 30 min in 5 mM aminophylline-Krebs solution containing different concentrations of cimetidine; histamine then was added. The curves show cyclic AMP accumulation after a 5-min incubation in histamine alone (●), histamine plus 3.5 μM cimetidine (○), histamine plus 35 μM cimetidine (△), and histamine plus 140 μM cimetidine (■). Each value represents the mean ± standard error ($n = 6$–12). Basal levels of cyclic AMP (shown at *B*) were not significantly altered by the different concentrations of cimetidine.

The *inset* depicts the Schild plot for cimetidine against histamine-induced cyclic AMP accumulation [least-squares regression of log(dose ratio − 1) versus log of the molar concentration of cimetidine]. Dose ratios were based on 10 concentration-response curves for histamine-induced cyclic AMP accumulation in the presence of three concentrations of cimetidine (3.5, 35, and 140 μM); each concentration-response curve was obtained on 15 preparations. The slope was 1.21 (0.93 to 1.45; 90% confidence limits), and the intersection with the abscissa gave the $pA_2$ value of 5.40.

without a significant decline in the maximal response to histamine.

Dose ratios based on the $EC_{50}$ values of histamine in the absence and presence of different concentrations of cimetidine were calculated. A Schild plot was constructed by plotting log(dose ratio − 1) versus log of the molar concentrations of cimetidine and fitting the points by least-squares linear regression. As shown in the *inset* of Fig. 4, the slope of the Schild plot for cimetidine against histamine was 1.21 (0.93–1.49, 90% confidence limits), a value which is not significantly different from unity. The calculated $pA_2$ value of cimetidine against histamine-induced cyclic AMP accumulation was 5.40.

The concentration-effect curve for histamine-stimulated cyclic AMP accumulation was unchanged in the presence of 1 μM propranolol, a *beta*-adrenoceptor antagonist. In the concentrations used, neither mepyramine, cimetidine, nor propranolol had a significant effect on the basal levels of cyclic AMP (Figs. 3 and 4).

*Effect of mepyramine, cimetidine, and propranolol on 2-thiazolylethylamine-stimulated cyclic AMP accumu-*

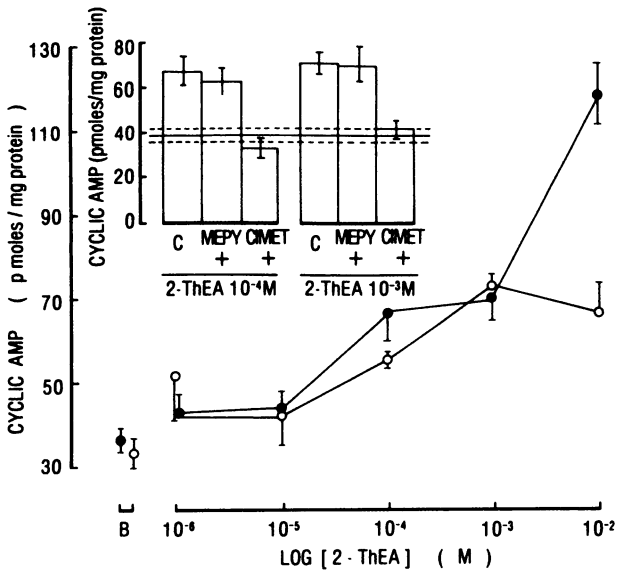


FIG. 5. *Effect of propranolol on 2-thiazolylethylamine-induced cyclic AMP accumulation in mouse vas deferens*

Tissues equilibrated for 1 hr in normal Krebs solution were incubated for 30 min in 5 mM aminophylline-Krebs solution containing 1 μM propranolol; 2-thiazolylethylamine then was added. The curves show cyclic AMP accumulation after a 5-min incubation in 2-thiazolylethylamine alone (●), $n = 6–9$, and 2-thiazolylethylamine plus 1 μM propranolol (○), $n = 3$. Each value represents the mean ± standard error. Basal levels of cyclic AMP in the two groups of tissues are represented at *B*.

The *inset* shows the effect of 1 μM mepyramine (*MEPY*) and 35 μM cimetidine (*CIMET*) on cyclic AMP accumulation elicited by $10^{-4}$ and $10^{-3}$ M 2-thiazolylethylamine (*2-ThEA*). The *solid horizontal line* indicates the mean basal level of cyclic AMP, and the *broken lines* indicate the standard error value derived from at least three tissues. Each histogram represents the mean ± standard error. The stimulation of cyclic AMP accumulation caused by each concentration of 2-thiazolylethylamine alone is represented at *C*.

*lation*. Unlike that caused by histamine, the maximal stimulation of cyclic AMP accumulation caused by 2-thiazolylethylamine was markedly reduced in the presence of 1 μM propranolol (Fig. 5). The stimulated increases in cyclic AMP accumulation elicited by $10^{-4}$ and $10^{-3}$ M 2-thiazolylethylamine were unaffected by 1 μM mepyramine but were abolished completely by 35 μM cimetidine (see *inset* of Fig. 5).

## DISCUSSION

This study shows that histamine stimulates cyclic AMP accumulation in isolated, intact mouse vas deferens, whether or not the tissues have been equilibrated in the presence of 5 mM aminophylline for an optimal period. However, the maximal accumulation of cyclic AMP was considerably greater in the presence of aminophylline. This finding indicates that the stimulatory effect of histamine on cyclic AMP levels in isolated, intact vas deferens is greatly masked by the rapid inactivation by the enzyme phosphodiesterase of cyclic AMP formed, an interpretation which is supported by the finding that the basal cyclic AMP level in the tissues was considerably lower in the absence than in the presence of aminophylline.

That the stimulatory effect of histamine on cyclic AMP accumulation was not antagonized by 0.1–1.0 μM mepyramine, an $H_1$ receptor antagonist, or by 1.0 μM propranolol, a *beta*-adrenoceptor antagonist, indicates that neither histamine $H_1$ receptors nor *beta*-adrenoceptors are involved in the mediation of histamine effect. However, the histamine stimulation of cyclic AMP accumulation was progressively depressed by increasing concentrations of cimetidine, an $H_2$ receptor antagonist. In the presence of different concentrations (3.5–140 μM) of cimetidine, the concentration-effect curve for histamine was displaced to the right in a parallel manner without a significant decline in its maximal response, indicating a competitive inhibition of the response to histamine. The latter conclusion was further confirmed by the calculated slope of the Schild plot and its 90% confidence limits, which were not significantly different from unity. The fact that the stimulatory effect of histamine on cyclic AMP accumulation was blocked by cimetidine and not by mepyramine indicates that this effect of histamine in mouse vas deferens is mediated via an $H_2$ receptor.

The $pA_2$ calculated in this study of cimetidine against histamine stimulation of cyclic AMP accumulation (5.40) is close to values reported previously (Table 1) against histamine inhibition of the electrically evoked twitch response in mouse vas deferens (5.05), rat vas deferens (5.47), and mouse seminal vesicle (5.77) (9–11). Although these $pA_2$ values of cimetidine in male accessory genital organs are similar to those reported by Angus *et al.* (25) for mouse isolated stomach preparations (5.14), they are lower than those (6.1 and 6.02) reported for other $H_2$ receptor-containing systems, such as guinea pig atrium and rat uterus (26). Whether these differences indicate heterogeneity in the histamine $H_2$ receptor or some unknown factor(s) interfering with the assay procedure(s) remains to be established. Recently, however, Angus and Black (27) examined the reliability of mouse stomach preparations for the assay of $H_2$ receptor antagonists and suggested that low $K_B$ values of the antagonists in this preparation were due to local tissue factors. Furthermore, the potency profiles of the agonists based on the pharmacological (inhibition of the electrically evoked twitch response) and biochemical (stimulation of cyclic AMP accumulation) criteria in mouse vas deferens were within the range of those reported for the well-characterized, typical $H_2$ receptors found in preparations such as the guinea pig atrium and rat uterus (Table 1).

Our finding that the 2-thiazolylethylamine-caused increase in cyclic AMP accumulation was blocked by cimetidine but not by mepyramine indicates that this effect of 2-thiazolylethylamine, an $H_1$ agonist, is mediated via stimulation of a cimetidine-sensitive $H_2$ receptor and not by stimulation of a mepyramine-sensitive $H_1$ receptor. That 2-thiazolylethylamine has a weakly stimulatory action on $H_2$ receptors is in agreement with the findings reported by several investigators (11, 28, 29). However, in contrast to these previous studies, the present findings indicate that 2-thiazolylethylamine probably acts as a partial agonist in stimulating cyclic AMP accumulation in mouse vas deferens preparations. Furthermore, the fact that the maximal stimulation by 2-thiazolylethylam-

TABLE 1

*Comparison of the pharmacological characteristics of the histamine $H_2$ receptor in mouse vas deferens with those of the histamine $H_2$ receptor reported in other preparations*

Abbreviations: ND, not determined; NA, not available; PA, partial agonist.

| Various organs and responses | Relative potencies of agonists | | | | | $pA_2$ values against histamine in antagonists | |
|---|---|---|---|---|---|---|---|
| | Histamine | Dimaprit | 4-Methylhistamine | 2-Methylhistamine | 2-Thiazolylethylamine | Cimetidine | Metiamide |
| Mouse vas deferens | | | | | | | |
| Cyclic AMP accumulation[a] | 100 | 64 | 31 | ND | PA | 5.40 | ND |
| Twitch response[b] | 100 | 26 | 18 | 3 | ND | 5.05 | 5.05 |
| Guinea pig atrium, chronotropic effect[c] | 100 | 71 | 43 | 4 | 0.5 | 6.10 | 6.04 |
| Guinea pig hippocampal slice, cyclic AMP accumulation[d] | 100 | 67 | 67 | 12 | 7 | 6.0 | 5.97 |
| Rat uterus, twitch response[e] | 100 | 18 | 25 | 2 | NA | 6.02 | 6.12 |
| Rat stomach, gastric secretion[f] | 100 | 20 | 39 | 2 | 0.3 | NA | 5.91 |

[a] Based on our present study.
[b] Taken from refs. 10 and 22.
[c] Taken from refs. 2, 4, and 23.
[d] Taken from ref. 24.
[e] Taken from refs. 2 and 4.
[f] Taken from refs. 2, 4, and 5.

ine of cyclic AMP accumulation was markedly depressed by 1 μM propranolol indicates that the stimulation of cyclic AMP accumulation produced by the high concentrations of the compound is partially secondary to the release of norepinephrine from the endogenous stores in mouse vas deferens. We have previously reported that 2-thiazolylethylamine produces contractions of rat isolated vas deferens by releasing norepinephrine from adrenergic nerves innervating the preparation (30). These findings in mouse and rat vas deferens, when considered together, strongly indicate that in high concentrations 2-thiazolylethylamine can produce additional effects which might be unrelated to its actions on either $H_1$ or $H_2$ (or both) receptors and that caution should therefore be exercised in the use of this agonist as a pharmacological tool for studies involving characterization of histamine actions or receptors.

Finally, this study shows that, in mouse vas deferens, the pharmacological properties of the histamine $H_2$ receptor which mediates increased cyclic AMP accumulation are similar to those of that which mediates histamine inhibition of the contractile response in the same preparation. The role of cyclic nucleotides in drug-induced smooth-muscle relaxation, although still quite controversial, is well documented (see references in ref. 31). Therefore, an obvious question arises as to what is the causal relationship between the increased cyclic AMP accumulation and the inhibition of the contractile response of the vas deferens caused by histamine. These studies are currently in progress.

## ACKNOWLEDGMENTS

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