therefore assessed 10 hr after drug administration in the following *in vivo* experiments.

Inhibition of MAO Activity. The procedures were similar to those of the *in vitro* studies except that animals were injected with four doses of inhibitors at a 0.25 log interval 10 hr before the brain and liver tissues were removed.

Effect on the Brain Amines. Elevations in the steady-state brain amines content were studied as follows. Groups of five mice weighing 16–25 g were injected at the same time of the day with the test compounds at 0.36 mmol/kg. The first control group received the vehicle (3% Tween 80). The second control group was given phosphate buffer, the vehicle for 3% Tween 80. The animals were decapitated and the brain was removed and frozen in Dry Ice until assayed. The rest of the procedures have been described previously.[20]

Prevention of Reserpine Ptosis. Groups of five mice weighing 16–25 g were pretreated with four doses of inhibitors at a 0.3 log interval. Reserpine (5 mg/kg) was administered 10 hr thereafter. Ptosis was considered significant if the opening of the palpebral fissure was not greater than 50% of normal. The dose which prevented ptosis from occurring 24 hr after injection of reserpine in 50% of the animals was calculated as the ED_{50}. Since reserpine-induced depletion of brain amines varies with the time of the day and follows a 24-hr rhythmic cycle,[21] the time of injection of reserpine and all inhibitors was kept as constant as possible.

Potentiation of *l*-Dopa Excitation. The procedures were similar to those of the preceding experiment except that *l*-Dopa (100 mg/kg) was injected instead of reserpine. The excitatory response to *l*-Dopa was rated by the method of Everett.[22] The dose which caused a 3+ excitation (piloerection, profuse salivation, markedly increased irritability, jumping, squeaking, and aggressive fighting) in 50% of the animals was calculated as the ED_{50}.

Analysis of Data. All LD_{50}, ED_{50}, and pI_{50} values were determined graphically by the probit method of Litchfield and Wilcoxon.[23] Multiple comparisons were made by Duncan's new multiple range test.[24] In several tests, the mean of the ED_{50} values and its standard error were calculated so as to minimize the variation in these values arising from different days of experiments with an inhibitor. All doses were calculated as the free base.

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Comparison of the Effects of Histamine and Tolazoline on Adenylate Cyclase Activity from Guinea Pig Heart

Ira Weinryb* and Inge M. Michel

Department of Biochemical Pharmacology, Squibb Institute for Medical Research, Princeton, New Jersey 08540.

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Both histamine and tolazoline (2-benzyl-2-imidazoline) stimulated particulate fractions of adenylate cyclase from guinea pig myocardium. Tolazoline was one-tenth as potent, and about two-thirds as active at maximally effective levels, as was histamine. Enhancement of cyclase activity by tolazoline was additive with that by isoproterenol, and the histamine and tolazoline concentration–response curves were parallel, suggesting that tolazoline acted at the same site as histamine. At maximally effective concentrations, tolazoline did not affect ATPase or cyclic AMP phosphodiesterase activities associated with the cyclase preparations. The H_1-receptor antagonist, mepyramine, and the H_2 antagonist, burimamide, blocked stimulation of cyclase by tolazoline at one-tenth the molarity of agonist. Both antagonists were less effective *vs.* histamine stimulation of heart cyclase in particulate fractions or whole homogenates, with mepyramine being generally more potent. It is suggested that the molecular basis of the stimulatory effect of tolazoline on cardiac tissue may be histaminergic stimulation of adenylate cyclase. Furthermore, the lack of potency of burimamide as a histamine antagonist and its lack of specificity compared to mepyramine, at the subcellular level, indicate that histamine-responsive adenylate cyclase from heart may not be a satisfactory molecular model for the H_2 receptor pharmacology of histamine in cardiac tissue.

In 1960, Trendelenburg[1] reported that histamine had direct stimulatory effects on isolated mammalian cardiac tissue and that these effects could not be blocked specifically by the classical antihistamines, mepyramine or tripelennamine. From a study of the relative activities of several histamine analogs on histamine-sensitive processes, Ash and Schild[2] differentiated histamine receptors into at least two types, those antagonized specifically by low concentrations of classical antihistamines (H_1) and those not sensitive to classical antihistamines. Black, *et al.*,[3] formally identified the latter class of effects of histamine as occurring at H_2 receptors and showed that burimamide [*N*-methyl-*N'*-[4-[4(5)-imidazolyl]butyl]thiourea] could competitively and specifically block the action of histamine at the H_2 receptor in isolated tissues, including guinea pig atrium. A new H_2-receptor antagonist,

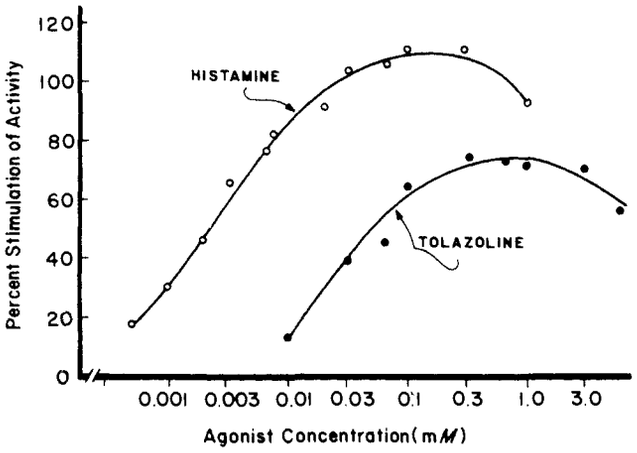

Figure 1. Concentration-response curves for the stimulation of adenylate cyclase activity from guinea pig myocardium by histamine and tolazoline. Cyclase activity in the absence of histamine was 314 pmol of cyclic AMP/mg of protein/15 min; that in the absence of tolazoline was 282 pmol of cyclic AMP/mg/15 min. Points on the histamine curve represent single assays; those on the tolazoline curve are the mean of duplicate assays.

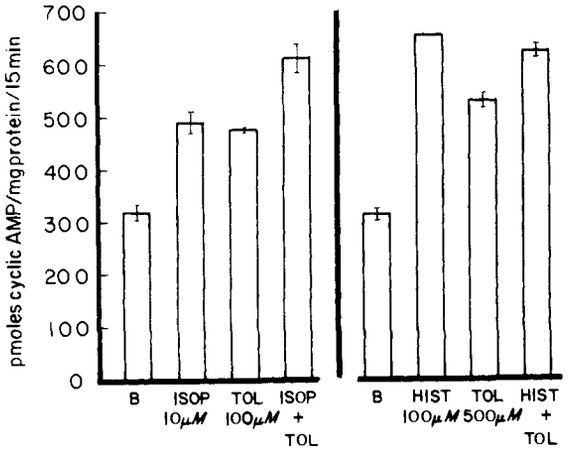

Figure 2. The effects of combination of isoproterenol (ISOP) and tolazoline (TOL), and of tolazoline and histamine (HIST) on heart cyclase basal (B) activity. Each bar shows the mean ± range of duplicate assays, except for the bar for 100 μM histamine, which represents a single assay.

metiamide [*N*-methyl-*N'*-[2-[(5-methylimidazol-4-yl)-methylthio]ethyl]thiourea], that is eight times as active as burimamide has recently been reported.[4]

The stimulatory effects of histamine on the isolated perfused guinea pig heart are accompanied by elevations in ventricular cyclic AMP levels; both the mechanical and biochemical effects of histamine are antagonized by burimamide.[5,6] As expected, histamine can also stimulate the activity of cardiac adenylate cyclase in subcellular fractions.[7,8] Tolazoline (2-benzyl-2-imidazoline) had also been known to stimulate cardiac tissue *via* a mechanism thought, from indirect evidence, to be sympathomimetic.[9] We show below that tolazoline acts at the histamine receptor to stimulate adenylate cyclase activity from heart and compare the ability of mepyramine and burimamide to antagonize stimulation of heart cyclase activity by histamine and tolazoline, with a view toward determining the utility of histamine-responsive cyclases from heart as subcellular models of the H_2-receptor complex.

Results

A comparison of the abilities of histamine and tolazoline to increase adenylate cyclase activity in a particulate fraction from guinea pig heart ventricular tissue is represented in Figure 1. Stimulation by histamine was detectable at 0.5 μM, half-maximal at 3 μM, and maximal at 100 μM. Tolazoline also stimulated cyclase at concentrations $\geq$10 μM; it was one-tenth as potent as histamine (half-maximal stimulation occurring at 30 μM) and approximately two-thirds as active at maximally effective levels (500 μM). The concentration-response curves for histamine and tolazoline were parallel, suggesting an action at a common receptor. Further experiments bearing on this possibility are summarized in Figure 2. Previous studies[7,8] had shown that stimulation of heart cyclase by maximally effective concentrations of β-adrenergic catecholamines and histamine was nearly or completely additive. The combination of tolazoline (100 μM) and a saturating concentration of isoproterenol (10 μM) produced nearly additive effects on cyclase activity. Also, the combination of saturating levels of histamine (100 μM) and tolazoline (500 μM) did not enhance cyclase activity above that observed in the presence of histamine alone. These results are also consistent with the concept that tolazoline stimulates a cyclase from heart *via* an interaction at the histamine receptor on the enzyme complex.

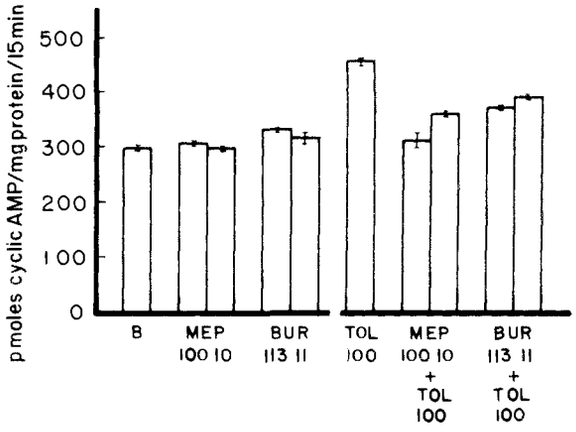

Figure 3. Antagonism of the stimulation of a previously frozen particulate fraction of heart cyclase induced by tolazoline (TOL), mepyramine (MEP), and burimamide (BUR). B refers to basal activity. Numbers associated with each bar represent the micromolar concentration of the corresponding agent. Each bar shows the mean ± range of duplicate assays.

The particulate fractions of adenylate cyclase employed here possess substantial ATPase and cyclic AMP phosphodiesterase (PDE) activities. We have ruled out inhibition of either ATPase or PDE activity, under the conditions of the cylase assay, as a possible cause of the apparent increase in cyclase activity in the presence of 100–1000 μM tolazoline (data not shown).

The ability of the H_1 antagonist, mepyramine, and the H_2 antagonist, burimamide, to block the stimulatory effects of tolazoline is detailed in Figure 3. Both antagonists were capable of significant blockade at one-tenth the molar concentration of agonist, mepyramine being perhaps somewhat more effective. Concentration-response curves for stimulation by tolazoline in the absence and presence of 10 μM burimamide or 10 μM mepyramine are presented in Figure 4. Mepyramine was consistently somewhat more effective than burimamide in shifting the concentration-response curve for tolazoline to the right in such experiments. Moreover, the antagonism seemed competitive in the sense that high concentrations of agonist appeared capable of overcoming inhibition by either antihistamine.

Surprisingly, mepyramine was a more effective antagonist than burimamide with histamine as agonist (Figure

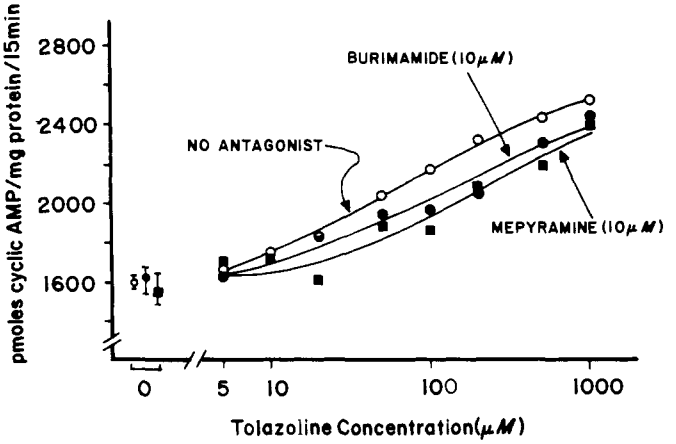

Figure 4. The concentration-response curve for the stimulation of heart cyclase activity by tolazoline in the absence (○) and presence of 10 μM burimamide (●) or mepyramine (■). Activities in the absence of tolazoline represent the mean ± range of five (○) or three (●, ■) determinations, respectively. The other points are individual assays. This experiment was carried out with three different cyclase preparations with similar results.

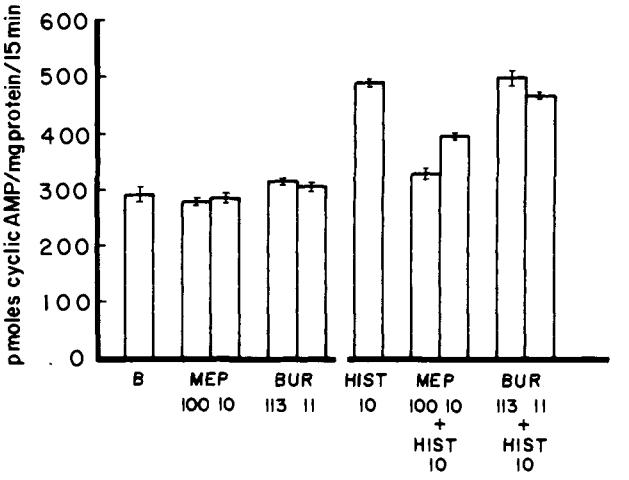

Figure 5. Antagonism of the stimulation of a previously frozen particulate fraction of heart cyclase induced by histamine (HIST), mepyramine (MEP), and burimamide (BUR). B denotes basal activity. Numbers associated with each bar are micromolar concentrations. Each bar shows the mean ± range of duplicate assays.

5), although neither blocking agent appeared as effective, on a molar basis, as with tolazoline as stimulator. These results, obtained with frozen cyclase fractions, were replicated with a freshly prepared crude homogenate and a fresh particulate fraction derived therefrom. Concentration-response curves for the antagonism of the effects of histamine by 10^{-5} M burimamide or mepyramine are depicted in Figure 6. A small but consistent shift of the concentration-response curve for histamine was observed in the presence of 10^{-5} M burimamide; again, mepyramine appeared to be a more effective antagonist. Linear transformation of these data indicated that the nature of the antagonism was either competitive or mixed (competitive-noncompetitive) but, in any event, did not distinguish between the two antihistamines.

Discussion

The investigations presented above have documented the ability of tolazoline to increase the activity of particulate fractions of an adenylate cyclase from guinea pig myocardium and have strongly indicated that tolazoline acts at the same site on the enzyme complex as does histamine. We suggest that the molecular basis of the stimulatory effects of tolazoline on cardiac tissue may be a histaminergic interaction with an adenylate cyclase.

Tolazoline had been reported to inhibit brain phosphodiesterase activity at very high concentrations (10 mM);[10] we found no effect of tolazoline at concentrations as high as 1 mM on phosphodiesterase activity in our heart cyclase preparations. Tolazoline (1 mM) can also increase the formation of cyclic AMP from labeled precursor in slices of cerebral cortex from guinea pig and rabbit, although it is only one-fifth and $\frac{1}{75}$th as effective as equimolar histamine, respectively.[11] It is not known whether tolazoline can stimulate histamine-responsive cyclases from tissues other than heart and cerebral cortex.

Our studies of the antagonism by burimamide of the action of histamine on preparations of guinea pig heart cyclase are not in quantitative agreement with recently published work by Verma and McNeill.[12] They reported that the potency of histamine as agonist was reduced by approximately a factor of ten in the presence of 10 μM burimamide. More importantly, we cannot find any evidence, at the subcellular level, for specificity of inhibition by burimamide relative to the H_1 antagonist, mepyramine. Burimamide has been considered a specific H_2 antagonist of the cardiac effects of histamine on the basis of experiments with atrial tissue or the whole perfused heart in which (1) burimamide acted as a competitive antagonist of the effect of histamine[3,6] and (2) burimamide did not show antiadrenergic, anticholinergic, or classical (H_1) antihistaminic properties at effective concentrations.[3] However, excess burimamide appeared to be necessary for substantial antagonism of the effects of histamine in cardiac tissue.[3,5,6] At the subcellular level, where the problems of tissue permeability, distribution, and metabolism were much less important, and where the toxicity of the classical antihistamines[5,8] did not prevent a direct comparison with burimamide, the latter compound was qualitatively not distinguishable from mepyramine in our hands and was a weak receptor antagonist compared to, for example, propranolol, which inhibits the stimulation of catecholamine-responsive heart cyclase by *saturating* concentrations of isoproterenol (10 μM) by 50% at $\frac{1}{50}$th the concentration of agonist (I. Weinryb and I. M. Michel, unpublished experiments). Given the apparent specificity of burimamide as an H_2 antagonist in tissue, our results may be due to (1) the presence of both H_1 and H_2 receptors in

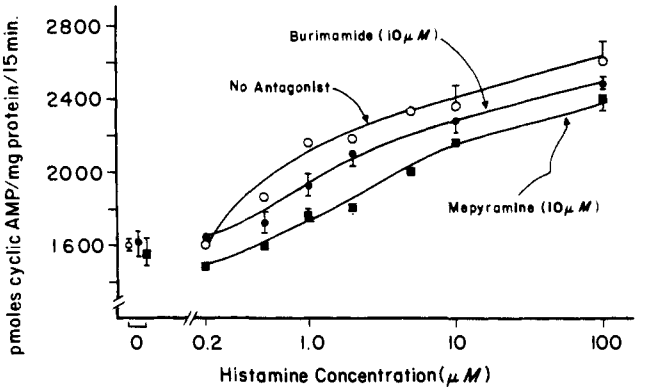

Figure 6. The concentration-response curve for the stimulation of heart cyclase activity by histamine in the absence (○) and presence of 10 μM burimamide (●) or mepyramine (■). Activities in the absence of histamine represent the mean ± range of five (■) or three (●, ■) determinations, respectively. The other points represent either the mean ± range of duplicate assays or individual assays. This experiment was carried out with three different cyclase preparations with similar results.

myocardium, a situation reflected in our enzyme fractions, or (2) the lability of the H_2 receptor to the procedures involved in obtaining the particulate enzyme preparations.

It is also possible that a more potent H_2-receptor antagonist than burimamide might allow a distinction between H_1 and H_2 antagonists at the subcellular level. Dousa and Code[13] have reported that stimulation by histamine of guinea pig gastric mucosal cyclase is blocked significantly by metiamide concentrations only 1% that of histamine. However, since they did not compare metiamide with an H_1 antihistamine in these studies, no definite conclusions about the uniqueness of action of metiamide *vs.* this enzyme preparation can be drawn.

The lack of potency of burimamide and its lack of specificity relative to mepyramine as a histamine receptor antagonist at the level of cardiac adenylate cyclase activity suggest that such enzyme complexes from myocardium may not be satisfactory molecular models for the H_2-receptor pharmacology of histamine in cardiac tissue.

Experimental Section

Enzyme Preparation. The preparation of guinea pig myocardial adenylate cyclase fractions has been previously described.[14] Briefly, the enzyme preparation is a once-washed particulate fraction derived from a 20% homogenate of ventricular tissue in $MgCl_2$ (1 m*M*)–glycylglycine (2 m*M*) buffer (pH 7.5), by centrifugation at 1000g for 15 min at 4°. Aliquots (0.5 ml) of the washed and resuspended pellets from the centrifugation were sealed in ampoules and stored under liquid nitrogen for future use. The results of Figures 4 and 6 were obtained with a particulate fraction, suspended in 10 m*M* Tris (pH 7.5),[12] that was used immediately after preparation.

Assay of Enzyme Activities. The assay for adenylate cyclase activity was essentially that reported by us previously.[14,15] [α-^{32}P]-ATP was the substrate and the isolation procedure for cyclic [^{32}P]-AMP included the use of cyclic [^{3}H]-AMP as a recovery standard, Dowex 50 chromatography, and $BaSO_4$ treatment. The basic assay system included 1.8 m*M* $MgCl_2$, 0.8 m*M* glycylglycine, 32 m*M* Tris (pH 7.8), 1.2 m*M* ATP, 5×10^6–10×10^6 cpm [α-^{32}P]-ATP, (50 μl) particulate enzyme fraction (125–225 μg of enzyme protein), 1 m*M* cyclic AMP, and test compound(s) in a total volume of 0.59 ml. ATPase activity was measured as previously outlined.[15] Cyclic AMP phosphodiesterase activity was determined by measuring the degradation of [^{3}H]-cyclic AMP in a cyclase assay mixture without ATP and in the absence and presence of 1 m*M* unlabeled cyclic AMP. Apparent cyclase activity was approximately linear with time in the absence and presence of histamine and tolazoline.

Materials. Isoproterenol (Schwartz/Mann) and histamine (Matheson Coleman and Bell) were obtained commercially. Tolazoline (Ciba-Geigy), mepyramine (Merck), and propranolol (Ayerst) were from the Squibb Institute chemical collection. Burimamide was a gift from Dr. J. W. Black (Smith Kline & French Laboratories, Ltd.). Other assay reagents were generally of analytical grade.

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Synthesis and Microbiological Activities of Some Monohalogenated Analogs of Tyrosine

Tommy J. McCord,* David R. Smith, Douglas W. Winters, John F. Grimes, Karen L. Hulme, Lawrence Q. Robinson, Larry D. Gage, and Alvie L. Davis

Department of Chemistry, Abilene Christian College, Abilene, Texas 79601. *Received August 5, 1974*

2-Chlorotyrosine and 2-bromotyrosine, as well as the previously reported 2-fluorotyrosine, were synthesized by hydrolysis of the condensation products from the appropriate benzyl bromide and ethyl acetamidomalonate and were compared with the corresponding 3-halotyrosines as growth inhibitors of *Escherichia coli* 9723, *Streptococcus faecalis* 8043, and *Lactobacillus plantarum* 8014. In contrast to the 2- and 3-fluorotyrosines which were equally effective as growth inhibitors, the 2-chloro- and 2-bromotyrosines were much more effective than the 3-chloro- and 3-bromotyrosines in inhibiting the growth of the three microorganisms. For each of the assay organisms, the growth inhibitions of all three 2-halotyrosines were reversed competitively in varying degrees by tyrosine.

Numerous studies on the preparation and biological activities of the various 3-monohalogenated and 3,5-dihalogenated tyrosines have been published. The general importance of these compounds results from their interrelationship to thyroxine biochemistry,[1] their isolation from natural sources,[2] and their abilities to act as metabolic inhibitors of biological systems.[3-5] On the other hand, only one of the four 2-halogenated tyrosines, 2-fluorotyrosine,[6] has been synthesized and virtually no study on its biological properties has appeared. Thus, the preparation of additional 2-halogenated analogs of tyrosine for microbiological studies was undertaken in the present study. Two new analogs of tyrosine, 2-chlorotyrosine and 2-bromotyrosine, were synthesized and studied together with 2-fluorotyrosine for growth-inhibiting properties in *Escherichia coli* 9273, *Streptococcus faecalis* 8043, and *Lactobacillus plantarum* 8014.

Chemistry. All three of the 2-halotyrosines 7–9 were