from fresh hog kidneys and assayed as reported in the literature.[14] Dopa (50 nmol) was incubated with 0.5 unit of AADC in a 0.5-ml incubation mixture and the evolved ^{14}C-labeled carbon dioxide trapped in NCS solubilizer and counted in a toluene cocktail with a 94% efficiency.

B. **Tyrosine Hydroxylase Assay.** Tyrosine hydroxylase (TH) was isolated from bovine adrenals by the method of Nagatsu, *et al.*[15] Its activity was determined by the coupled decarboxylase assay[14] as modified previously.[1] $DMPH_4$ was dissolved in 0.005 *N* HCl and the inhibitors were dissolved in the phosphate buffer. An excess of AADC (7.5 units per 0.5-ml incubation mixture) was used to decarboxylate the Dopa-*carboxyl*-^{14}C formed from tyrosine-*carboxyl*-^{14}C. Under these conditions no limitation on decarboxylation was observed even with compounds which in the assay of AADC were good inhibitors.

C. **Dopamine β-Hydroxylase Assay.** Dopamine β-hydroxylase (DBH) was isolated from bovine adrenal medullary tissue as described by Friedman and Kauman[16] and modified by Kuzuya and Nagatsu.[17] It was assayed by the spectrophotometric method reported in the literature.[12]

Acknowledgments. This investigation was supported by a U. S. Public Health Service Research Grant No. HE-11274 from the National Institutes of Health. The authors wish to express their appreciation to Professors Paul A. Weinhold and Matthias C. Lu for their interest in this work. We are also grateful to Ronald Langdon for his technical assistance in certain phases of this study.

References

(1) L. E. Hare, M. C. Lu, C. B. Sullivan, P. T. Sullivan, R. E. Counsell, and P. A. Weinhold, *J. Med. Chem.*, 17, 1 (1974) (paper 3).
(2) S. Udenfriend, *Pharmacol. Rev.*, 18, 43 (1966).
(3) E. M. Johnson, Jr., N. Zenker, and J. Wright, *Biochem. Pharmacol.*, 21, 1777 (1972); K. E. Moore and J. A. Dominic, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 30, 859 (1971).
(4) S. Udenfriend, P. Zaltsman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, 14, 837 (1965).
(5) S. B. Barker in "Medicinal Chemistry," 2nd ed, A. Burger, Ed., Interscience, New York, N.Y., 1960, p 682.
(6) E. C. Jorgensen, *Annu. Rep. Med. Chem.*, 1965, 200 (1965).
(7) R. E. Counsell, P. Desai, A. Ide, P. G. Kulkarni, P. A. Weinhold, and V. B. Rethy, *J. Med. Chem.*, 14, 789 (1971).
(8) W. N. White and E. F. Wolfarth, *J. Org. Chem.*, 35, 2196 (1970).
(9) D. S. Tarbell, *Org. React.*, 2, 1 (1944).
(10) T. F. West, *J. Soc. Chem. Ind., London*, 59, 275 (1940).
(11) K. Biemann, "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York, N.Y., 1963, pp 544–567.
(12) C. R. Creveling, J. W. Daly, B. Witkop, and S. Udenfriend, *Biochem. Biophys. Acta*, 64, 125 (1962).
(13) J. M. Pepper and M. Saha, *Can. J. Chem.*, 42, 113 (1964), and modified according to the procedure of R. V. Heinzelman, "Organic Syntheses," Collect. Vol. IV, Wiley, New York, N.Y., 1963, p 573.
(14) J. C. Waymire, R. Bjur, and N. Weiner, *Anal. Biochem.*, 43, 588 (1971).
(15) T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, 239, 2910 (1964).
(16) S. Friedman and S. Kaufman, *J. Biol. Chem.*, 240, 4763 (1965).
(17) H. Kuzuya and T. Nagatsu, *Enzymologia*, 36, 31 (1969).

Effect of Acylation with Eleostearic Acids on the Monoamine Oxidase Inhibitory Potency of Some Hydrazine Antidepressants in Mice

Song Y. Hsu,*† Chian L. Huang, and Irving W. Waters

Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi 38677. *Received July 24, 1974*

The effect of incorporation of an eleostearoyl group into molecules of aralkylhydrazines on their monoamine oxidase inhibitory potency was investigated *in vitro* and *in vivo*. The results showed that on a molar basis the hydrazides possessed an *in vitro* potency lower than and an *in vivo* potency and acute toxicity comparable to those of the corresponding aralkylhydrazines. The sequence of the relative potency of aralkylhydrazines and their hydrazides was similar. The overall pharmacological profile indicated that these aralkylhydrazines retained their monoamine oxidase inhibitory properties when the free hydrazino nitrogen was acylated with an eleostearoyl group.

Pheniprazine (PIH), phenelzine (PEH), benzylhydrazine (BZH), and isopropylhydrazine are among the most potent monoamine oxidase (MAO) inhibitors,[1] yet only phenelzine has been used as a safe antidepressant. Acylation of these hydrazines with various acyl groups has led, in some instances, to hydrazides possessing comparable potency, greater organ specificity, and reduced toxicity.[1,2] The unsaturated long-chain fatty acids such as oleic, linoleic, and linolenic acid inhibit mitochondrial MAO to some extent *in vitro* whereas the saturated fatty acids such as palmitic and stearic acid are ineffective.[3] These fatty acids are taken up by the brain without prior oxidation to acetate.[4-6] This property would furnish a possibility of transport of the hydrazide molecules made from similar fatty acids and aralkylhydrazines to the brain for action. The present study was undertaken to determine whether these aralkylhydrazines retain their potency when the free hydrazino nitrogen is acylated with eleostearic acids. Syntheses of these hydrazides have been described previously.[7]

† Address correspondence to this author at the Department of Pharmacology, School of Medicine, State University of New York, Buffalo, N.Y. 14214.

Pharmacological Results. Acute Toxicity. Neither a lethal effect nor toxic symptoms other than diarrhea were observed for eleostearic acid isomers at 10.8 mmol/kg (3.0 g/kg) over a period of 2 weeks. The acute toxicity as indicated by LD_{50} over a 72-hr period of the hydrazides was equal to that of the corresponding aralkylhydrazines (Table I). The neuropharmacologic symptoms at 0.70 mmol/kg were sedation induced by benzylhydrazine and its hydrazide and central stimulation induced by phenelzine, pheniprazine, and their hydrazides. The central stimulant effects of the four inhibitors were qualitatively similar. The prominent symptoms included enhanced spontaneous motor activity, jumping and squeaking, and stereotyped behavior. The onset of central stimulation was 8–15 min after injection of phenelzine and pheniprazine and 2–2.5 hr after their acylated derivatives. It lasted 0.5–2 hr in the case of hydrazines, whereas it persisted for 5–7 hr in the case of hydrazides.

Inhibition of MAO Activity *in Vitro.* The inhibitory

Table I. Acute Toxicity of Hydrazine Derivatives in Mice

Compd	LD_{50}, mmol/kg ip (95% fiducial limits)
α-ESA[a]	10.8[b]
β-ESA	10.8
ESA-BZH	0.55 (0.47–0.65)
ESA-PEH	0.59 (0.49–0.71)
ESA-PIH	0.52 (0.40–0.67)
BZH	0.57 (0.48–0.68)
PEH	0.69 (0.53–0.90)
PIH	0.60 (0.49–0.75)

[a]For abbreviations, see the Experimental Section. [b]No lethal effect at the indicated dose level of both acid isomers.

effect on the brain MAO of aralkylhydrazines and their acylated derivatives followed a similar sequence of relative potency: pheniprazine > benzylhydrazine > phenelzine ($p < 0.01$) and acylated pheniprazine > acylated benzylhydrazine > acylated phenelzine ($p < 0.01$) (Table II). Their effect on the liver enzyme showed a similar sequence except for acylated pheniprazine > acylated benzylhydrazine ($p < 0.01$) = acylated phenelzine. As regards the effect on the enzyme of both tissues, the acylated derivatives of benzylhydrazine, phenelzine, and pheniprazine were slightly less potent than the parent aralkylhydrazines ($p < 0.05$). The two fatty acid isomers had only a slight effect.

Inhibition of MAO Activity *in Vivo.* In contrast to their *in vitro* effect, the acylated aralkylhydrazines were as potent as aralkylhydrazines in inhibiting the brain enzyme *in vivo* (Table II). There existed the same sequence of order of potency on the brain enzyme for these hydrazines and hydrazides ($p < 0.05$) as was observed *in vitro.* However, all inhibitors were equally potent in inhibiting the liver enzyme *in vivo.*

Elevation of Brain Amines. Most of the results of these experiments were reported previously.[7] Table III presents the effects of phosphate buffer and benzylhydrazine which were not published. In summary, all treatments caused no change in DA levels. The vehicle (3% Tween 80 in phosphate buffer) did not affect brain amine content. The hydrazides were as potent as their parent aralkylhydrazines with regard to their effect on NE and 5-HT levels. The relative potency of aralkylhydrazines was pheniprazine > benzylhydrazine ($p < 0.01$) = phenelzine for their ability to increase the levels of both amines. The hydrazides exhibited the same sequence for their effect on 5-HT ($p < 0.01$) and were equally effective in increasing NE content. α-Eleostearic acid elevated NE and 5-HT levels slightly.

Prevention of Reserpine-Induced Ptosis. The ptotic effect of reserpine always occurred in conjunction with other symptoms such as sedation, tremor, and increased muscular tone. All these symptoms produced at 5 mg/kg were prevented by pretreatment of MAO inhibitors. All inhibitors were equally effective against reserpine ptosis. This antagonistic effect was still detectable 2 weeks after injection of doses above the ED_{50}.

Potentiation of *l*-Dopa-Induced Excitation. *l*-Dopa at 300 mg/kg induced a 1+ excitation (piloerection, slight salivation, slightly increased motor activity). A dose of 100 mg/kg which produced no sign of central stimulation when injected alone precipitated a 1+ to 3+ response in the animals pretreated with inhibitors in a dose-related manner. Table IV shows the ED_{50} of all hydrazine derivatives to elicit a maximal degree (3+) of potentiation. The excitatory effect occurred immediately after the injection of *l*-Dopa and persisted for 0.5–1.5 hr. All inhibitors were equally potent. The duration of the detectable potentiating effect was longer than 2 weeks at doses above the ED_{50}.

Discussion

Toxicity is one of some major problems associated with the therapeutic use of alkyl- and aralkylhydrazine MAO inhibitors as antidepressants. Acylation of these hydrazines has produced isocarboxazide and nialamide as effective antidepressants with reduced toxicity. It is conceivable that the effort to reach a potential therapeutic agent by this approach would begin with the comparison of MAO inhibitory potency between the acylated derivatives and the parent hydrazines. The present paper represents such an initial effort.

Using an established statistical procedure of multiple comparisons, it was possible to demonstrate that in all *in vivo* studies introducing an eleostearoyl group into the molecules of aralkylhydrazines conferred, on a molar basis, on the resulting hydrazides a comparable potency on the brain and liver MAO. It was also noted that three acylated aralkylhydrazines possessed a sequence of order of potency similar to that of the parent hydrazines with regard to their *in vivo* inhibitory effect on the brain MAO and their ability to increase 5-HT levels in the brain.

Table II. Inhibition of Monoamine Oxidase by Hydrazine Derivatives

	$pI_{50} \pm$ S.E.[a] (in vitro)		$ED_{50} \pm$ S.E.,[b] μmol/kg ip	
Compd	Brain	Liver	Brain	Liver
α-ESA	3.9 ± 0.1	4.0 ± 0.2		
β-ESA	3.9 ± 0.1	3.8 ± 0.1		
ESA-BZH	5.2 ± 0.1	6.0 ± 0.2	6.6 ± 0.4	5.5 ± 0.5
ESA-PEH	5.3 ± 0.1	5.4 ± 0.1	6.9 ± 0.2	13.0 ± 1.5
ESA-PIH	6.4 ± 0.1	7.1 ± 0.2	6.5 ± 0.4	3.0 ± 0.6
BZH	6.3 ± 0.1	7.0 ± 0.2	6.5 ± 0.8	6.2 ± 0.4
PEH	5.7 ± 0.1	6.2 ± 0.1	7.1 ± 1.0	10.0 ± 1.0
PIH	7.1 ± 0.2	8.0 ± 0.2	7.3 ± 0.6	2.0 ± 0.2

[a]Inhibitors of varying concentrations (10^{-2}–10^{-7} *M*) were incubated with solubilized mitochondrial MAO of mice at 37° for 8 min prior to the addition of *m*-iodobenzylamine (7.2×10^{-4} *M*) in phosphate buffer. The pI_{50} is the negative logarithm of the concentration required for 50% inhibition of the enzyme activity. Each value represents the mean ± standard error of five determinations. [b]The mean ± standard error of five experiments.

Table III. Effect of Hydrazine Derivatives on the Brain Amine Levels in Mice[a]

	μg/g of wet tissue		
Compd	DA[b]	NE	5-HT
Phosphate buffer	0.879 ± 0.032	0.465 ± 0.008	0.609 ± 0.028
3% Tween 80	0.883 ± 0.035	0.466 ± 0.004	0.613 ± 0.012
ESA-BZH	0.886 ± 0.034	0.982 ± 0.003	1.403 ± 0.038
BZH	0.874 ± 0.042	0.958 ± 0.030	1.386 ± 0.049

[a] Each value represents the mean ± standard error for a group of three to five mice 10 hr after administration of inhibitors at 0.36 mmol/kg. [b]DA for dopamine, NE for norepinephrine, and 5-HT for 5-hydroxytryptamine.

Table IV. Antagonism of Reserpine Ptosis and Potentiation of *l*-Dopa Excitation in Mice

Compd	$ED_{50} \pm S.E.$[a] (mmol/kg ip)	
	Prevention of reserpine ptosis[b]	Potentiation of *l*-Dopa excitation[c]
α-ESA	Ineffective	Ineffective
β-ESA	Ineffective	Ineffective
ESA-BZH	0.09 ± 0.03	0.11 ± 0.03
ESA-PEH	0.12 ± 0.02	0.08 ± 0.02
ESA-PIH	0.12 ± 0.02	0.07 ± 0.02
BZH	0.14 ± 0.04	0.08 ± 0.03
PEH	0.16 ± 0.03	0.08 ± 0.01
PIH	0.18 ± 0.03	0.05 ± 0.02

[a]Each value represents the mean ± standard error of four experiments. [b]Reserpine, 5 mg/kg ip. [c]*l*-Dopa, 100 mg/kg ip.

Since, in addition to the inhibition of MAO, these aralkylhydrazines are able to inhibit dopamine β-hydroxylase to a different extent[8] and since there exists a possibility that they may also be able to release amines to a different degree from their storage sites in the brain judging from the fact that pheniprazine is capable of such an action,[9-11] the effect of aralkylhydrazines and their hydrazides on the brain amine levels may be the resultant of interactions among these actions. Such interactions could also account for the equal effect of these inhibitors on the brain NE levels and in other *in vivo* tests.

The present finding that all inhibitors were devoid of an effect on the endogenous DA content is consistent with the previous data.[12] It is well known that MAO inhibitors are capable of elevating DA content markedly in the brain of the *l*-Dopa-treated animals. This could be explained by a recent finding that hydrazine inhibitors are relatively ineffective against "DA MAO."[13] It is conceivable that for a given extent of inhibition of DA MAO with these inhibitors, the endogenous-free DA may be still normally oxidized by the enzyme, whereas the elevated-free DA derived from *l*-Dopa treatment may not be effectively oxidized. This possibility is suggested by the fact that a rise in brain 5-HT becomes detectable only when MAO activity is inhibited by 85% or more.[14] Thus, a partial, inadequate inhibitory action on the DA MAO of hydrazine inhibitors would cause no effect on the endogenous DA content but would be able to increase it after *l*-Dopa treatment.

The *in vitro* studies on the enzyme of brain and liver showed that the potency of the hydrazides was slightly lower than that of the parent hydrazines. Accumulating evidence indicates that the MAO inhibitory effect of hydrazides may be mediated by monosubstituted hydrazines released from the molecules of hydrazides by a hydrolytic enzyme hydrolase.[15-17] The inferior effect of the hydrazides could most probably be due to inadequate biotransformation in the *in vitro* biological system. Also, the necessity of the hydrolytic release of the effective hydrazine moiety may at least in part account for the much slower onset of central stimulant effect of the acylated derivatives of phenelzine and pheniprazine.

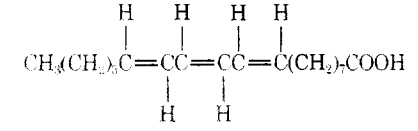
In agreement with the previous finding that higher unsaturated fatty acids inhibit mitochondrial MAO to various extent *in vitro*,[3] eleostearic acid isomers exerted a slight effect on the brain MAO *in vitro* and *in vivo*. However, their effect appeared of no significance in contributing to the effect of hydrazides since the molar potency of hydrazides was equal to that of the parent aralkylhydrazines.

Although acylation with eleostearic acids of aralkylhydrazines did not reduce the LD_{50} values of their acylated derivatives on a molar basis, their chronic toxicity remains to be studied before conclusions can be drawn as to whether the acylated derivatives are safer MAO inhibitor antidepressants.

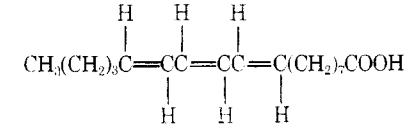
It has long been known that phenelzine and pheniprazine produce a central stimulant effect in animals. In the present studies, the incorporation of an eleostearoyl group into these molecules did not interfere with this effect qualitatively. However, the onset and duration of this effect were prolonged. This property points to a possibility, which is yet to be investigated, that these acylated derivatives could be more effective inhibitors by chronic administration.

Experimental Section

Drugs. The compounds investigated were α-eleostearic acid (α-ESA, 1), β-eleostearic acid (β-ESA, 2), *N*-benzyl-α-eleostearoyl hydrazide (ESA-BZH, 3), *N*-phenethyl-β-eleostearoyl hydrazide (ESA-PEH, 4), *N*-α-methylphenethyl-β-eleostearoyl hydrazide (ESA-PIH, 5), benzylhydrazine dihydrochloride (Aldrich Chemical Co., Milwaukee, Wis.), phenelzine sulfate (Warner-Chilcott Laboratories, Morris Plains, N.J.), and pheniprazine hydrochloride (Lakeside Laboratories, Milwaukee, Wis.). The numbered compounds were prepared in this laboratory.[7] Reserpine and *l*-Dopa (Sigma Chemical Co., St. Louis, Mo.) were used in the drug interaction tests.



1. α isomer



2. β isomer

α-RCONHNHCH$_2$C$_6$H$_5$ (3) β-RCONHNHCH$_2$CH$_2$C$_6$H$_5$ (4)

β-RCONHNHCH(CH$_3$)CH$_2$C$_6$H$_5$ (5)

R = CH$_3$(CH$_2$)$_3$(CH=CH)$_3$(CH$_2$)$_7$–

Administration of Drugs. All test compounds were either dissolved or emulsified in 3% Tween 80 in phosphate buffer, 0.05 *M*, pH 7.6. Reserpine (5 mg) was dissolved in 0.1 ml of glacial acetic acid and diluted to 10 ml with distilled water. *l*-Dopa (100 mg) was dissolved in 0.8 ml of 2.4 *N* HCl and diluted to 10 ml with distilled water. All injections were made intraperitoneally to male albino mice (SAF/ICR, Southern Animal Farms, Prattville, Ala.).

Acute Toxicity. The acute LD_{50} in 72 hr was determined for each compound in five groups of eight mice weighing 25–30 g. A dose-ranging test was carried out to obtain the optimum four doses at a 0.1 log interval to be used for the final assay. With respect to ESA which did not cause any deaths in 72 hr at 1.0 g/kg, doses as high as 3.0 g/kg were tested.

Studies *in Vitro*. Inhibition of MAO Activity. The MAO inhibitory effect was studied *in vitro* on the brain and liver mitochondrial MAO. The pooled brain and liver tissues of five to ten mice weighing 16–25 g were processed by the same procedures[18] to obtain mitochondria. The activity of the enzyme was assayed by a spectrophotometric method.[19]

Studies *in Vivo*. In preliminary experiments where the animals were pretreated with inhibitors at doses near the ED_{50} and were challenged with a subsequent dose of *l*-Dopa at 100 mg/kg, the maximal potentiating effect of inhibitors was observable 6–20 hr after injection. For comparison, the potency of all inhibitors was

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.

References

(1) J. H. Biel, A. Horita, and A. E. Drukker, "Psychopharmacological Agents," Vol. I, M. Gordon, Ed., Academic Press, New York, N. Y., 1964, p 359.
(2) J. H. Biel, *Advan. Chem. Ser.*, **No. 45**, 144 (1964).
(3) E. A. Rapava, L. B. Klyashtorin, and V. Z. Gorkin, *Biokhimiya*, **31**, 1216 (1966).
(4) G. A. Dhopeshwarkar and J. F. Mead, *Biochim. Biophys. Acta*, **187**, 461 (1969).
(5) G. A. Dhopeshwarkar and J. F. Mead, *Biochim. Biophys. Acta*, **210**, 250 (1970).
(6) G. A. Dhopeshwarkar, C. Subramanian, and J. F. Mead, *Biochim. Biophys. Acta*, **231**, 8 (1971);**239**, 162 (1971).
(7) S. Y. Hsu, C. L. Huang, and I. W. Waters, *J. Med. Chem.*, **16**, 450 (1973).
(8) B. M. Bloom, *Ann. N. Y. Acad. Sci.*, **107**, 878 (1963).
(9) J. Glowinski, M. Hamon, F. Javoy, and Y. Morot-Gaudry, *Advan. Biochem. Psychopharmacol.*, **5**, 423 (1972).
(10) J. Knoll and K. Magyar, *Advan. Biochem. Psychopharmacol.*, **5**, 393 (1972).
(11) N. Weiner and R. Bjur, *Advan. Biochem. Psychopharmacol.*, **5**, 409 (1972).
(12) H. Corrodi, *J. Pharm. Pharmacol.*, **18**, 197 (1966); K. E. Moore, *ibid.*, **20**, 656 (1968).
(13) M. B. H. Youdim, *Brit. Med. Bull.*, **29**, 120 (1973).
(14) K. F. Gey and A. Pletscher, *J. Neurochem.*, **6**, 239 (1961).
(15) M. A. Schwartz, *J. Pharmacol. Exp. Ther.*, **135**, 1 (1962).
(16) B. A. Koechlin, M. A. Schwartz, and W. E. Oberhaensli, *J. Pharmacol. Exp. Ther.*, **138**, 11 (1962).
(17) T. Satoh and K. Moroi, *Biochem. Pharmacol.*, **21**, 3111 (1972).
(18) H. R. Mahler and E. H. Cordes, Ed., "Biological Chemistry," Harper and Row, New York, N.Y., 1966, p 394.
(19) V. Zeller, G. Ramachander, and E. A. Zeller, *J. Med. Chem.*, **8**, 440 (1965).
(20) N. H. Neff, P. F. Spano, A. Groppetti, C. T. Wang, and E. Costa, *J. Pharmacol. Exp. Ther.*, **176**, 701 (1971).
(21) I. B. Black, L. Parker, and J. Axelrod, *Biochem. Pharmacol.*, **18**, 2688 (1969).
(22) G. M. Everett, "Antidepressant Drugs," S. Garattini and N. N. G. Dukes, Ed., Excerpta Medica Foundation, Amsterdam, 1966, p 164.
(23) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(24) D. B. Duncan, *Biometrics*, 11, 1 (1955).

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.

Received May 30, 1974

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,