creatic elastase. Enzyme solutions preincubated without drugs and solutions of drugs alone were included in the elastase and esterase assays for control purposes. Siliconized glassware was used throughout these procedures.

Hydrocortisone, phenylbutazone and indomethacin, in concentrations ranging from 0·1 to 1·0 mg/ml and in two different buffer systems (described in Table 1), failed to inhibit elastolysis by leukocyte granules in my experiments. Phenylbutazone and indomethacin were also ineffective against esterolysis of NBA by granule extracts. Preincubation times were 15 min in the case of the hydrocortisone and ranged from 15 min to 3 hr in the case of the nonsteroidal agents. The same results were obtained with acetylsalicylic acid, except that the latter was tested in concentrations of 0·01 to 0·1 mg/ml. On the other hand, gold salt proved to be an effective inhibitor of both elastolysis and esterolysis by leukocyte granules. The results of experiments with this agent are summarized in Table 1.

The concentration range of gold salt used to demonstrate inhibition in these experiments was approximately 5×10^{-4} to 5×10^{-3} M. It is unlikely that gold compounds reach this concentration in synovial or other tissue fluids of patients treated with the drug. However, such concentrations may be attained within the lysosomes of leukocytes which have phagocytosed and sequestered gold salts within their cytoplasmic granules. Thus, an effective concentration of the inhibitor could be reached at the sites of enzyme storage in inflammatory cells.

In conclusion, five anti-inflammatory agents were tested for possible inhibition of the elastolytic and esterolytic activities of human leukocyte granules. Hydrocortisone, acetylsalicylic acid, phenylbutazone and indomethacin were ineffective. Of the compounds tested, only gold sodium thiomalate possessed inhibitory activity against these granule-mediated reactions.

Department of Pathology AARON JANOFF*
New York University School of Medicine,
New York, N.Y., U.S.A.

* Career Development Award Fellow of the United States Public Health Service (GM-K3-6461).

REFERENCES

1. R. H. PERSELLIN and M. ZIFF, *Arthritis Rheum.* **9**, 57 (1966).
2. R. S. ENNIS, J. L. GRANDA and A. S. POSNER, *Arthritis Rheum.* **11**, 756 (1968).
3. J. PROKOPOWICZ, *Thromb. Diath. haemorrh.* **19**, 84 (1968).
4. A. JANOFF and J. D. ZELIGS, *Science, N.Y.* **161**, 702 (1968).
5. M. ZIFF, H. J. GRIBETZ and J. LOSPALLUTO, *J. clin. Invest.* **39**, 405 (1960).
6. A. JANOFF and J. SCHERER, *J. exp. Med.* **128**, 1137 (1968).
7. G. S. LAZARUS, J. R. DANIELS, R. S. BROWN, H. A. BLADEN and H. M. FULLMER, *J. clin. Invest.* **47**, 2622 (1968).
8. L. A. SACHER, K. K. WINTER, N. SICHER and S. FRANKEL, *Proc. Soc. exp. Biol. Med.* **90**, 323 (1955).
9. L. VISSER and E. R. BLOUT, *Fedn Proc.* **28**, 407 (1969).
10. A. JANOFF, *Biochem. J.* **114**, 157 (1969).

Biochemical Pharmacology, Vol. 19, pp. 628–632. Pergamon Press. 1970. Printed in Great Britain

Effects of hydroxamic acids on L-histidine carboxy-lyase*

(*Received* 9 *June* 1969; *accepted* 25 *July* 1969)

THE HYDROXYLAMINE derivative, 4-bromo-3-hydroxybenzyloxyamine (NSD-1055), is a potent inhibitor of L-histidine carboxy-lyase (histidine decarboxylase, HD),[1] and reduces tissue histamine levels in

* Aided by Grant GM-13958 from the National Institutes of Health, United States Public Health Service.

TABLE 1. EFFECTS OF HYDROXYLAMINE AND CERTAIN HYDROXAMIC ACIDS ON L-HISTIDINE CARBOXYLYASE FROM TWO SOURCES*

Compound	Inhibition at 10^{-4}M (%)	
	Mammalian enzyme	Bacterial enzyme
Hydroxylamine	100	88
OHA	91	31
APHA	96	23
HHA	10	67

*Reaction mixtures were as follows: Mammalian enzyme—L-histidine-^{12}C ($7{\cdot}5 \times 10^{-4}$M), pyridoxal phosphate (10^{-5}M), DL-histidine-^{14}C (0·375 μCi), and enzyme solution (0·3 ml) in a total volume of 0·53 ml of 0·1 M potassium phosphate buffer, pH 6·9, at 37°. The reaction was initiated by addition of enzyme. After incubation for 30 min the reaction was terminated by adding 0·5 ml of 1·2 N H_2SO_4. Bacterial enzyme—as above except the reaction volume was 2·2 ml of 0·2 M potassium citrate, pH 4·5, at 37°, 0·02 μCi of DL-histidine-^{14}C was used, 1·0 mg of enzyme was added in 1·0 ml, and pyridoxal phosphate was omitted.

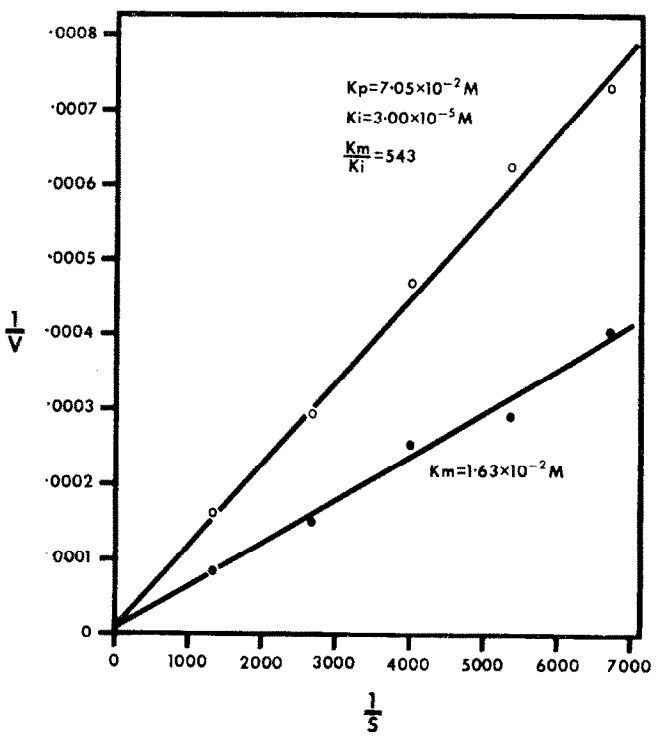

FIG. 1. Competitive inhibition of bacterial HD by HHA. ●, Control; ○, 10^{-4}M HHA. Reaction conditions were the same as described for Table 1 except for variations in substrate and isotope concentrations. Lines were placed and kinetic data were calculated by the method of least squares.

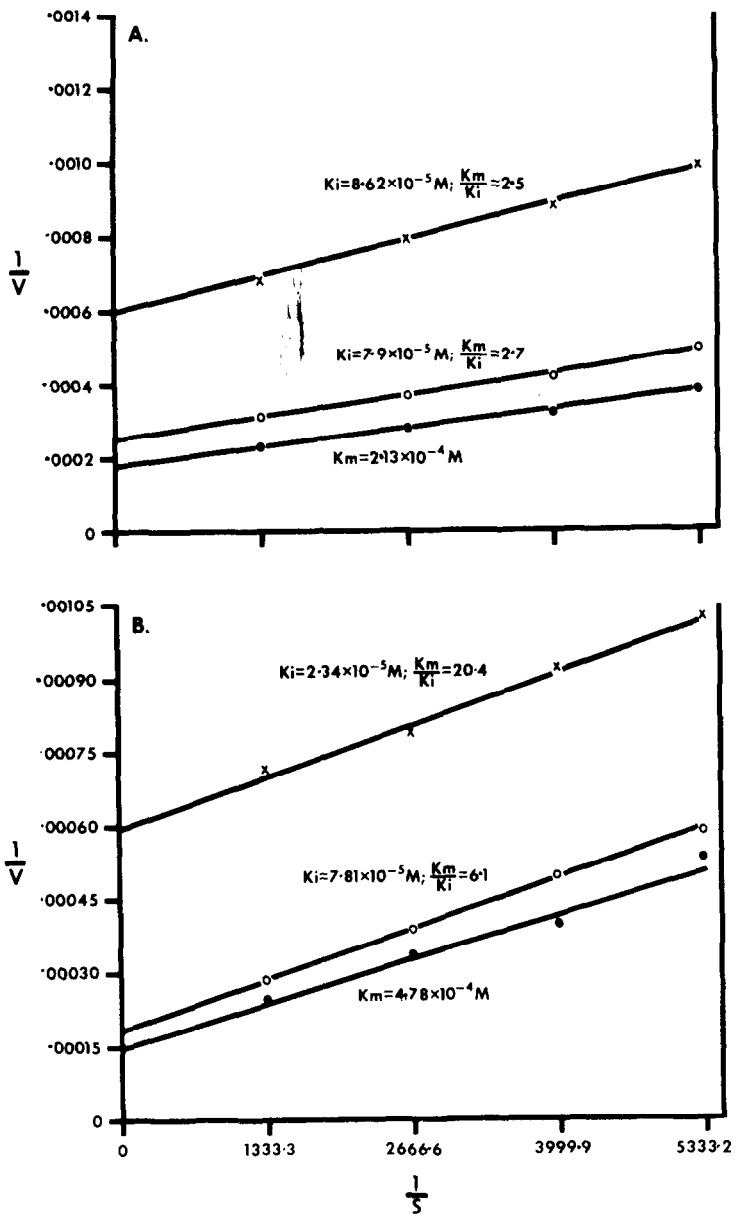

FIG. 2. Uncompetitive or coupling inhibition of mammalian HD by (A) OHA and (B) APHA. Reaction conditions were the same as described for Table 1 except for variations in substrate, isotope, and inhibitor concentrations. A: ●, control; ○, 1·5 × 10^{-5}M OHA; ×, 5·5 × 10^{-5}M OHA. B: ●, control; ○, 1·5 × 10^{-5}M APHA; ×, 6·0 × 10^{-5}M APHA. Lines were placed and kinetic data were calculated by the method of least squares.

rats.[2] Kinetic data, when using the enzyme from *Clostridium welchii*, reveal a mixed type of inhibition[3] while the enzyme from rat fetus is competitively inhibited.[4] A survey of activity in a limited group of closely related congeners demonstrated a striking dependence of activity upon the 4-bromo-3-hydroxy-positions; i.e. the 2-bromo-3-hydroxy- and the 5-bromo-3-hydroxy-analogs are only 1 per cent or less as active as NSD-1055.[5] The work reported herein was initiated to determine if representatives of another class of hydroxylamine derivative, hydroxamic acids, possess inhibitory action against this enzyme from two sources.

Bacterial HD was obtained from Nutritional Biochemicals Corp. and was used without further purification. Rat fetuses were obtained from Pel-Freez Biologicals, Inc., Rogers, Ark., and the enzyme was purified as described by Leinweber.[4] Hydroxamic acids were synthesized by Dr. J. B. Hynes, School of Pharmacy, Medical College of South Carolina, or obtained from various commercial sources. The assay mixture for the fetal enzyme was as described by Leinweber,[4] using DL-histidine-carboxyl-^{14}C from New England Nuclear Corp. With both enzymes, evolved $^{14}CO_2$ was trapped on KOH-impregnated filter paper strips and measured as described in an earlier report.[6]

A total of 84 hydroxamic acids and closely related derivatives was tested at 10^{-4}M against both enzymes. Hydroxylamine was included in numerous experiments as a positive control and consistently yielded a high degree of inhibition of both enzymes. Only those hydroxamates which conferred 60 per cent or greater inibition at this concentration were studied in some detail. Only 3 of the eighty-four compounds were found to possess this degree of activity: oxamylhydroxamic acid (OHA), 3-amino-pyrazino-2-hydroxamic acid (APHA), and L-histidylhydroxamic acid (HHA). A selectivity of action against each of the two enzyme species is implied as shown in Table 1; however, this may be only a reflection of the degree of dissociation of each compound at the two hydrogen ion concentrations. Plots of percentage inhibition against the logarithm of the OHA and APHA concentrations were linear, and the calculated concentration which conferred 50 per cent inhibition was $2{\cdot}6 \times 10^{-5}$M for both compounds. Most of the compounds were virtually totally inactive at the concentration used, yielding only 1–10 per cent inhibition. A few were moderately stimulatory, increasing the reaction rate 10–20 per cent above the control rate.

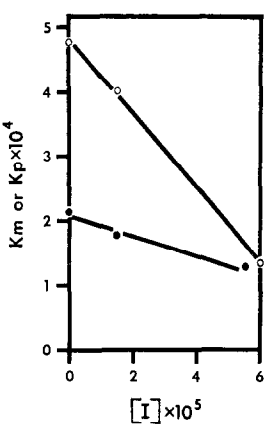

FIG. 3. Apparent dissociation constants of mammalian HD as a function of inhibitor concentration. ●, OHA; ○, APHA.

Double reciprocal plots of the data obtained when inhibitor was held at a constant concentration and substrate was varied showed that inhibition of bacterial HD by HHA was competitive in nature (Fig. 1). The value of K_p, the apparent dissociation constant in the presence of inhibitor, was 4·3 times that of K_m, and the ratio K_m/K_i indicated a relatively high affinity for the substrate binding site.

Similar treatment of the data obtained with the fetal enzyme using OHA and APHA resulted in plots which were virtually parallel, typical of the relatively uncommon uncompetitive or coupling inhibition in which the inhibitor is bound by the enzyme–substrate complex only, and not by the free enzyme (Fig. 2, A and B). A similar uncompetitive inhibition by 2-hydrazinopyridine of bacterial HD

has been reported.[8] The resulting linear decrease in K_p values as a function of inhibitor concentration is shown in Fig. 3. Values of K_i at the two OHA concentrations were in good agreement, whereas somewhat more variation was found with K_i values of APHA. The ratios K_m/K_i indicated a quite low order of affinity of the inhibitor for the enzyme–substrate complex.

The mammalian enzyme was used in experiments to assess the capacity of excess pyridoxal phosphate (PyP) to overcome inhibition conferred by OHA and APHA. Double reciprocal plots of the data so obtained in the presence of each inhibitor were consistently nonlinear and were not satisfactorily interpretable. There was evidence, however, that the coenzyme at least partially antagonizes the action of each hydroxamate. OHA is known to bind PyP as evidenced by a marked alteration of the ultraviolet absorption spectrum of the coenzyme;[7] however, no such alteration could be demonstrated in the presence of APHA.

Veterans Administration Hospital and
Department of Pharmacology,
Medical University of South Carolina,
Charleston, S.C., U.S.A.

GLEN R. GALE
ALAYNE B. SMITH
LORETTA M. ATKINS

REFERENCES

1. W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **237**, 89 (1962).
2. R. J. LEVINE, T. L. SATO and A. SJOERDSMA, *Biochem. Pharmac.* **14**, 139 (1965).
3. E. N. PERKINSON and J. P. DAVANZO, *Biochem. Pharmac.* **17**, 2498 (1968).
4. F. -J. LEINWEBER, *Molec. Pharmac.* **4**, 337 (1968).
5. L. ELLENBOGEN, E. MARKLEY and R. J. TAYLOR, JR., *Biochem. Pharmac.* **18**, 683 (1969).
6. J. A. HOWLE and G. R. GALE, *Proc. Soc. exp. Biol. Med.* **131**, 697 (1969).
7. G. R. GALE, *Cancer Res.* **26**, 2340 (1966).

Biochemical Pharmacology, Vol. 19, pp. 632–636. Pergamon Press. 1970. Printed in Great Britain

(3-Bromo-2-oxopropyl) trimethylammonium bromide, an inhibitor of acetylcholinesterase

(*Received* 29 *April* 1969; *accepted* 11 *July* 1969)

WITH the objective of developing an alkylating type of active site reagent for acetylcholinesterase (AChE; acetylcholine acetylhydrolase, EC 3.1.1.7), (3-bromo-2-oxopropyl)trimethylammonium bromide* and several structurally related compounds were examined. These include: 1-(3-bromo-2-oxopropyl)pyridinium bromide, 3-(bromoacetyl)-1-methylpyridinium bromide, (3-bromo-4-oxopentyl)trimethylammonium bromide and 2-[chloro(hydroxyimino)methyl]-1-methylpyridinium chloride. Of the group, BAT showed the most promise. (a) It reversibly inhibits the enzyme if assay is performed rapidly and shortly after mixing. (b) It progressively and irreversibly inhibits the enzyme, upon incubation, in a time-, concentration- and pH-dependent fashion. (c) Progressive inhibition is slowed by the reversible AChE inhibitor, tetramethylammonium bromide (TMA), and is prevented by prior diethyl phosphorylation. These results suggest that BAT may have utility as an active site reagent for AChE.

* Named 3-bromoacetonyltrimethylammonium bromide by Persson *et al.*[1] For convenience, we will refer to this compound as BAT.