Laboratory of Medicinal Chemistry and Pharmacology
Developmental Therapeutics Program
Division of Cancer Treatment
National Cancer Institute
Bethesda, MD 20205, U.S.A.

SUSAN SOMFAI-RELLE
KAYOKO SUZUKAKE
BARBARA P. VISTICA
DAVID T. VISTICA*

* Address all correspondence to: David T. Vistica, Ph.D., Laboratory of Medicinal Chemistry and Pharmacology, DTP, DCT, National Cancer Institute, Bldg. 37, Rm. 6D28 Bethesda, MD 20205.

REFERENCES

1. K. Suzukake, B. J. Petro and D. T. Vistica, *Biochem. Pharmac.* **31**, 121 (1982).
2. K. Suzukake, B. P. Vistica and D. T. Vistica, *Biochem. Pharmac.* **32**, 165 (1983).
3. O. W. Griffith and A. Meister, *J. biol. Chem.* **254**, 7558 (1979).
4. M. Chu and G. A. Fischer, *Biochem. Pharmac.* **17**, 753 (1968).
5. O. W. Griffith, *Analyt. Biochem.* **106**, 207 (1980).
6. E. Boyland and L. F. Chasseaud, *Biochem. J.* **104**, 95 (1967).
7. P. G. Richman, M. Orlowski and A. Meister, *J. biol. Chem.* **248**, 6684 (1973).
8. R. L. Beamer, O. W. Griffith, J. D. Gass, M. E. Anderson and A. Meister, *J. biol. Chem.* **255**, 11732 (1980).

Biochemical Pharmacology, Vol. 33, No. 3, pp. 490–493, 1984
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Mechanism of metallic mercury oxidation *in vitro* by catalase and peroxidase

(*Received* 21 *March* 1983; *accepted* 21 *July* 1983)

Kudsk [1] found that ethyl alcohol inhibited the uptake of metallic mercury by blood *in vitro* and *in vivo*. Ogata *et al.* [2] described the oxidation of metallic mercury by human red blood cells having different catalase activities, hypocatalasemia and acatalasemia, with or without hydrogen peroxide. The peroxidase in animals occurs as glutathione peroxidase in red blood cells, as myeloperoxidase in white blood cells, and as lactoperoxidase in milk, while the peroxidase in plants is of the horseradish type. Horseradish peroxidase is reported by Ikeda *et al.* [3] to be capable of oxidizing metallic mercury. Lactoperoxidase, which is very similar to horseradish peroxidase in regard to oxidizing phenols and aromatic amines in the presence of hydrogen peroxide, was used in experiments reported in this paper. The present authors recently demonstrated [4] the oxidation of metallic mercury by lactoperoxidase.

In experiments *in vivo* with acatalasemia mice exposed to metallic mercury, the oxidation rates of metallic mercury in the lung and blood were shown to be greater than those in normal mice, with increased levels also seen in the liver [5]. The results were considered as indicating that a peroxidase, other than catalase, in the liver is possibly involved in the oxidation of metallic mercury. Ogata *et al.* [6] demonstrated previously, through the use of proteins such as lactoperoxidase and ferritin, that the oxidation of metallic mercury by blood is so related.

The present report deals with the mechanism of oxidation of metallic mercury *in vitro* by the peroxidative enzymes, catalase and lactoperoxidase or horseradish peroxidase, using ethyl alcohol, L-dopa and pyrogallol as auxilliary substrates in the presence of hydrogen peroxide.

Crystalline bovine liver catalase (2x crystallized, 33,900 Sigma units/mg protein) and lactoperoxidase from cow's milk (79 units/mg protein using the pyrogallol method) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Horseradish peroxidase (100–150 units/mg protein) was obtained from the Wako Chemical Co., Osaka, Japan. All other reagents, including ethyl alcohol, L-β-(3,4-dihydroxyphenyl)alanine (L-dopa), pyrogallol, and hydrogen peroxide, were of analytical grade.

The reaction mixture was placed in the main chamber of a 15.ml Warburg flask with 0.1 ml of metallic mercury or metallic mercury vapor generated by the addition of stannous chloride to mercuric chloride in the side arm and 0.1 ml of hydrogen peroxide in the center well. Phosphate buffer was added to give a final volume of 3 ml. Incubation was conducted at 37° for 90 min with shaking at 80 cycles/min. The amount of mercury present in the main chamber after incubation was determined by an elemental mercury analyzer (Hitachi, model 207) with circulating air which contained mercury vapor, as described in our previous report [7].

The oxidation of metallic mercury by bovine liver catalase in the presence of hydrogen peroxide tended to increase with increased activity of catalase [8]. When ethyl alcohol was added, as an inhibitor of catalase activity and metallic mercury oxidation, to the catalase–H_2O_2 system at a fixed concentration of hydrogen peroxide, the oxidation of metallic mercury decreased as the concentration of ethyl alcohol was increased. The result is shown in Table 1. The oxidation of metallic mercury by catalase in the absence of ethyl alcohol was 1.23 μg/ml of incubation mixture. The competitive nature of the inhibition by ethyl alcohol was shown by Lineweaver–Burk plots [9], with two straight lines having different slopes with or without ethyl alcohol and crossing at the same intercept on the axis (Fig. 1). The result suggests that the reacting sites on the catalase for mercury and ethyl alcohol are very close to each other, or identical.

The oxidation of metallic mercury by lactoperoxidase increased with increased concentration of hydrogen peroxide [10] under the experimental conditions employed. When L-dopa was added to the lactoperoxidase–H_2O_2 system, the oxidation of metallic mercury by lactoperoxidase at a fixed concentration of hydrogen peroxide decreased as the concentration of L-dopa was increased. The result is shown in Table 2. The L-dopa used in this experiment may have served as a substrate [11] and/or a reducing agent for lactoperoxidase. The Lineweaver–Burk plots for mercury oxidation by lactoperoxidase with or without L-dopa

Table 1. Effect of the concentration of ethyl alcohol on the oxidation *in vitro* of metallic mercury by catalase

Catalase*† (90 μg)	EtOH‡ (mM)	H_2O_2 (%)	Mercury oxidation (μg/ml)	Mercury oxidation (μg/μmole protein)
+	515	3	0.136 ± 0.003	360
+	51.4	3	0.226 ± 0.067	602
+	5.14	3	0.955 ± 0.012	2547
+	0.514	3	1.05 ± 0.06	2800
+		3	1.23 ± 0.05	3280

* The calculations of enzyme activity were based on a mol. wt of 240,000 for catalase; 90 μg corresponds to 3.8×10^{-4} μmole of catalase.
† Bovine liver catalase.
‡ Ethyl alcohol.

Table 2. Effect of the concentration of L-dopa on the oxidation *in vitro* of metallic mercury by lactoperoxidase*

Lactoperoxidase† (26 μg)	L-dopa‡ (mM)	H_2O_2 (%)	Mercury oxidation (μg/ml)	Mercury oxidation (μg/μmole protein)
+	16.7×10^{-1}	3	0.117 ± 0.003	417
+	16.7×10^{-2}	3	0.174 ± 0.051	621
+	16.7×10^{-3}	3	0.252 ± 0.018	901
+	16.7×10^{-4}	3	0.383 ± 0.024	1366
+		3	0.642 ± 0.098	2293

* The calculations of enzyme activity were based on a mol. wt of 93,000 for lactoperoxidase; 26 μg corresponds to 3.0×10^{-4} μmole of lactoperoxidase.
† Lactoperoxidase from milk.
‡ L-β-(3,4-Dihydroxyphenyl)alanine.

gave two straight lines with different slopes but with the same intercept on the axis (Fig. 2). The competitive nature of the result indicates that L-dopa and mercury served as substrates for lactoperoxidase.

When pyrogallol was added to the horseradish peroxidase–H_2O_2 system as its substrate, the oxidation of metallic mercury by the enzyme decreased with increased concentration of pyrogallol. The result is shown in Table 3. The result was very similar to the oxidation of metallic mercury by lactoperoxidase. The Lineweaver–Burk plots for horseradish peroxidase gave a competitive inhibition with respect to pyrogallol and mercury (Fig. 3); both may have served as substrates for horseradish peroxidase reaction.

Schonbaum and Chance [12] proposed catalase compound I, an enzyme–peroxide complex, for peroxide-dependent oxidations of lower aliphatic alcohols, etc. Concerning the underlying mechanism of oxidation of mercury

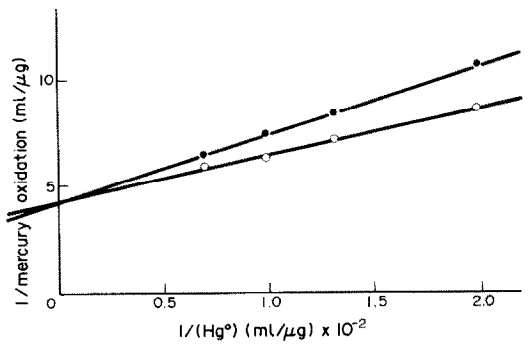

Fig. 1. Double-reciprocal plots of mercury oxidation by bovine liver catalase with and without ethyl alcohol. Key: (●) 53 mM ethyl alcohol, and (○) without added ethyl alcohol.

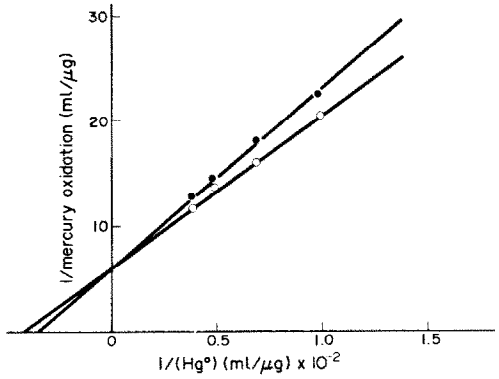

Fig. 2. Double-reciprocal plots of mercury oxidation by lactoperoxidase with and without L-dopa. Key: (●) 16.7×10^{-3} mM L-dopa, and (○) without added L-dopa.

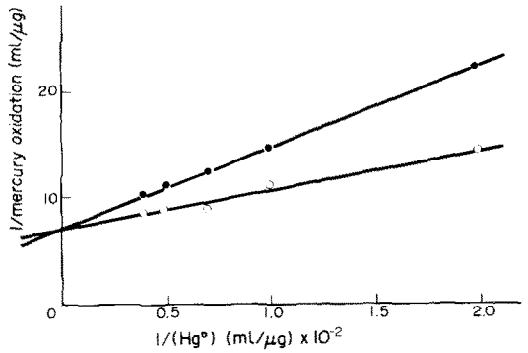

Fig. 3. Double-reciprocal plots of mercury oxidation by horseradish peroxidase with and without pyrogallol. Key: (●) 16.7×10^{-3} mM pyrogallol, and (○) without added pyrogallol.

by catalase, the authors propose the following equations based on the hypothesis of Schonbaum and Chance [12] and Deisseroth and Dounce [13]:

or simply

$$[\text{E-Fe}^{3+}\text{—OH}]\ \text{H}^{+} = \text{E-Fe}^{3+} + \text{H}_2\text{O} \quad (1)$$

$$\text{E-Fe}^{3+} + \text{HOOH} \text{—} \text{E-Fe}^{3+}\text{—O} + \text{H}_2\text{O} \quad (2)$$

$$\text{E-Fe}^{3+}\text{—O—}[\text{E-Fe}^{5+}\text{=O=E-Fe}^{5+}\text{—O}^{-}] \quad (3)$$
Comp. I

$$\text{E-Fe}^{5+}\text{=O} + \text{H}_2\text{O}_2 \text{—} \text{E-Fe}^{3+} + \text{H}_2\text{O} + \text{O}_2 \quad (4)$$

$$\text{E-Fe}^{5+}\text{=O} + \text{Hg}^{\circ} \text{—} \text{E-Fe}^{4+} + \text{HgO} \quad (5)$$

$$\text{E-Fe}^{5+}\text{=O} + \text{CH}_3\text{CH}_2\text{OH} \text{—} \text{E-Fe}^{3+} + \text{CH}_3\text{CHO} + \text{H}_2\text{O} \quad (6)$$

(E-Fe^{+} indicates Enzyme-tyr-O-Fe^{-})

The reaction mechanism of catalase with ethyl alcohol also can be depicted as shown by Schonbaum and Chance [12]. Equations 5 and 6 indicate the competition of compound I with metallic mercury and ethyl alcohol. In regard to peroxidase reactions, L-dopa or pyrogallol may play a role similar to that of ethyl alcohol.

In a previous report [5], we examined the *in vitro* mercury oxidation by liver homogenates obtained from animals pretreated with aminotriazole. The ratio of *in vitro* mercury oxidation by liver homogenates of normal, acatalasemia.

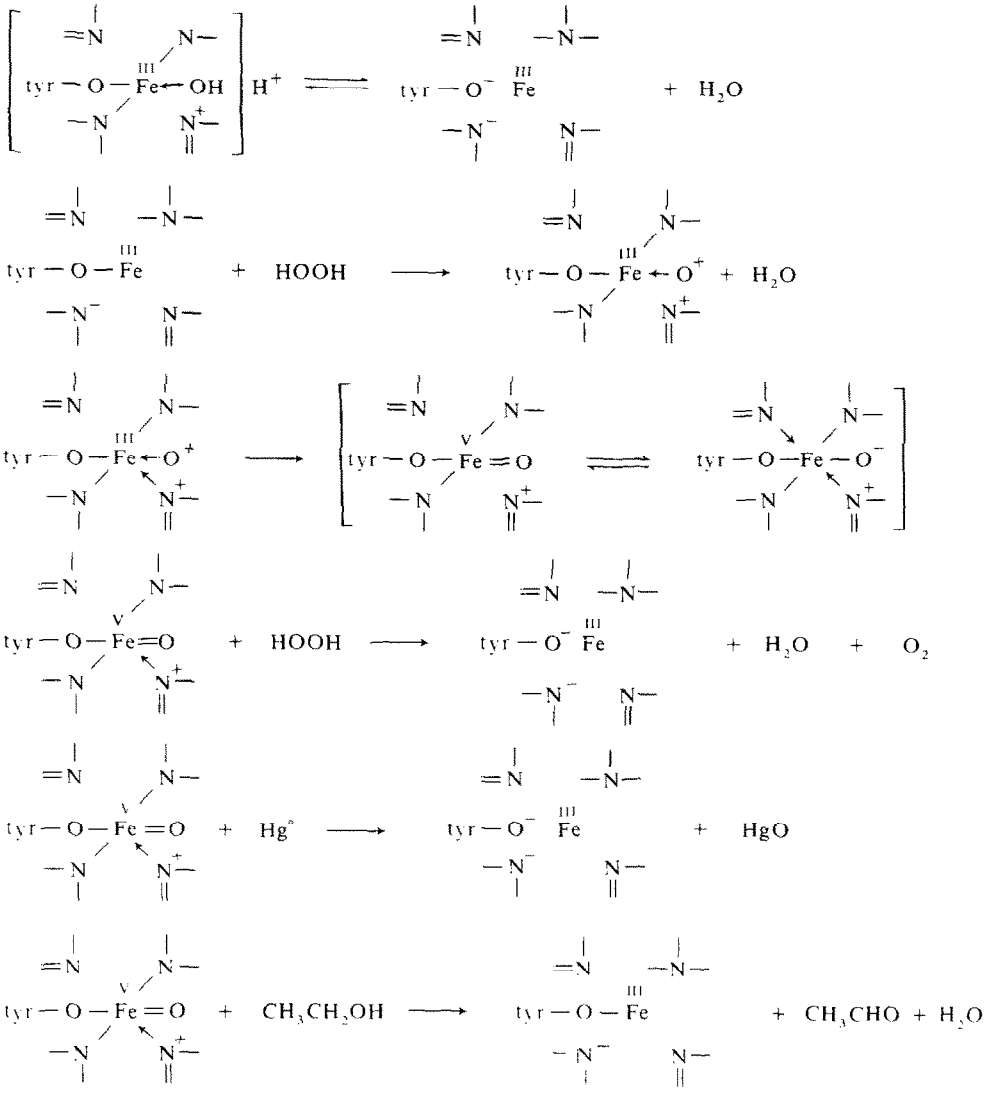

Table 3. Effect of the concentration of pyrogallol on the oxidation *in vitro* of metallic mercury by horseradish peroxidase (HRP)*

HRP (26 μg)	Pyrogallol (mM)	H_2O_2 (%)	Mercury oxidation (μg/ml)	(μg/μmole protein)
+	16.7×10^{-1}	3	0.091 ± 0.012	155
+	16.7×10^{-2}	3	0.093 ± 0.009	157
+	16.7×10^{-3}	3	0.222 ± 0.027	376
+	16.7×10^{-4}	3	0.464 ± 0.032	784
+		3	0.541 ± 0.096	915

* The calculations of enzyme activity were based on a mol. wt of 44,000 for horseradish peroxidase; 26 μg corresponds to 6.0×10^{-4} μmole of horseradish peroxidase.

normal treated with aminotriazole, and acatalasemia treated with aminotriazole was 100:84:74:39 without hydrogen peroxide and 113:91:73:57 with hydrogen peroxide while the ratio of their catalase activities was 100:40:9:0.2.

The effects of ethanol and pyrogallol on mercury oxidation are under investigation.

Acknowledgements—The authors express their thanks to Dr. Kazuhisa Taketa, Dr. Toshihiko Ubuga, Dr. Shinji Ohmori and Dr. Mikiko Ikeda for help in preparing this manuscript.

Department of Public Health
Okayama University Medical School
Okayama City, 700 Japan

MASANA OGATA*
HIROMI AIKOH

REFERENCES

1. F. N. Kudsk, *Acta pharmac. tox.* **27**, 149 (1969).
2. M. Ogata, K. Kenmotsu, N. Hirota and M. Naito, *Jap. J. ind. Hlth* **23**, 172 (1981).
3. M. Ikeda, K. Kumashiro, B. Inouc, M. Ogata and T. Ishida, *Ann. Hyg. Lab. Okayama Prefecture* No. 1, 104 (1977).
4. M. Ogata and H. Aikoh, *Ind. Hlth* **20**, 71 (1982).
5. M. Ogata and M. Ikeda, *Int. Archs occup. environ. Hlth* **41**, 87 (1978).
6. M. Ogata, K. Kenmotsu, N. Hirota and H. Aikoh, *Archs Toxic.* **50**, 93 (1982).
7. M. Ogata and H. Aikoh, *Ind. Hlth* **19**, 211 (1981).
8. K. Kenmotsu, *Okayama-Igakkai. Zasshi* **92**, 999 (1980).
9. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56**, 658 (1934).
10. H. Aikoh, *Okayama-Igakkai-Zasshi* **94**, 629 (1982).
11. B. D. Polis and H. W. Shmukler, *J. biol. Chem.* **201**, 475 (1953).
12. G. R. Schonbaum and B. Chance, *Catalase: The Enzymes* (Ed. P. D. Boyer), 3rd Edn, Vol. 13, p. 363. Academic Press, New York (1976).
13. A. Deisseroth and A. L. Dounce, *Physiol. Rev.* **50**, 319 (1970).

* Author to whom all correspondence should be addressed.

Biochemical Pharmacology, Vol. 33, No. 3, pp. 493–496, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Interaction of lisuride with monoamine receptors on human blood platelets

(*Received* 30 *May* 1983; *accepted* 2 *September* 1983)

The ergot derivative lisuride, a simple semisynthetic 6-methyl-isoergolene derivative with a urea residue in position 8, has been introduced into clinical practice for treatment of migraine [1]. Moreover, it inhibits prolactin secretion and is used as an antiparkinsonian drug [2, 3]. Biochemical and pharmacological studies have shown that lisuride interacts with dopamine and serotonin receptors as well as with adrenoceptors [1, 3–7]. Direct binding studies indicate that ^{3}H-lisuride is bound specifically to α_2-adrenoceptors, dopamine$_2$- and high affinity serotonin receptors in CNS [8].

Since blood platelets are a particularly useful tool for studying aminergic reactions [9, 10], they were used to examine the influence of lisuride on monoamine receptors. Blood platelets possess receptors for catecholamines and serotonin which differ from the carrier for the active amine uptake [9, 11–13]. Interaction of catecholamines or serotonin with specific platelet receptors produces biochemical and morphological changes of platelets resulting in shape change and/or aggregation and release reaction [9, 12, 14]. We studied the influence of lisuride on the adrenaline-induced and serotonin- or dopamine-potentiated, ADP-induced aggregation. By comparison with the selective α_2-adrenoceptor blocking agent rauwolscine, the serotonin receptor antagonist pizotifen and the dopamine receptor antagonist haloperidol we attempted to demonstrate the specific effect of lisuride. Furthermore, we examined to what extent lisuride interferes with the ^{3}H-yohimbine binding in intact platelets.

Materials and Methods

The following substances were used: (−) Adrenaline bitartrate (VEB Jenapharm, GDR); serotonin: 5-hydroxytryptamine creatinine sulphate (Merck, F.R.G.); lisuride