Biochemical Pharmacology, Vol. 21, pp. 3305–3308. Pergamon Press, 1972. Printed in Great Britain.

SHORT COMMUNICATIONS

Catalase involvement in microsomal ethanol-oxidizing system

(*Received* 28 *April* 1972; *accepted* 14 *July* 1972)

ALTHOUGH the hepatic microsomal ethanol-oxidizing system (MEOS)[1,2] has been suggested as an alternative to the alcohol dehydrogenase pathway, much evidence argues against a significant role of MEOS in ethanol metabolism *in vivo* (see Refs. 3 and 4). Indeed, it has been proposed that MEOS is not a distinct enzyme system analogous to the intact microsomal drug-metabolizing systems, but a fortuitous combination of H_2O_2 generation by microsomal NADPH-oxidase activity and subsequent oxidation of ethanol by the H_2O_2 plus catalase.[5–7]

The counter-argument, i.e. that catalase is not an essential participant in the MEOS activity, is based on the observation that various inhibitors produce substantially different degrees of effect on the catalase and MEOS activities of the same microsomal preparations.[8] For example, when microsomes were prepared from livers removed 23 hr after oral administration of a single dose of pyrazole (4·4 m-moles/kg), microsomal catalase activity was reduced by 90 per cent and ethanol oxidation supported by an H_2O_2-generating system (hypoxanthine plus xanthine oxidase) was reduced by 80 per cent, whereas the NADPH-dependent MEOS activity was decreased by only 16 per cent.[2] A similar but less striking difference in effects on the three systems was also observed with 10^{-4} M sodium azide.

This argument fails to take account of the fact that hepatic catalase activity is present in very large excess relative to the rate of H_2O_2 generation *in vivo*. In a reconstituted system, the degree of inhibition of the overall reaction caused by addition of a catalase inhibitor depends upon whether the enzyme or the supply of H_2O_2 is rate-limiting. We have explored this question with the MEOS and have found that, at appropriate rates of H_2O_2 generation, the rates of ethanol oxidation by H_2O_2-supported and NADPH-supported microsomal systems are reduced to virtually the same degree by catalase inhibitors.

Adult male Wistar rats, obtained from Canadian Breeding Laboratories, Montreal, were used in all experiments. They were allowed Purina chow and tap water *ad lib*. For preparation of microsomes, the animals were decapitated, the livers removed and the 105,000 *g* pellet was prepared as described previously.[6] Microsomal protein was measured by the biuret method.[9] In experiments in which several different times of incubation were employed, sufficiently large samples were obtained by pooling livers from four rats for each preparation. In other experiments, individual preparations were used.

Three different enzymatic activities were studied in these preparations. Catalase was assayed by the perborate method of Feinstein.[10] NADPH-dependent MEOS activity was assayed as described previously.[6] Catalatic oxidation of ethanol was studied by replacing NADPH in the incubation mixture with a hypoxanthine/xanthine oxidase (HX/XO) system for H_2O_2 generation.[8] In different experiments, xanthine oxidase was used at concentrations of 0·01, 0·02, 0·5 and 1·0 unit/ml of incubation mixture.

The effect of pyrazole was studied by administration of the drug (4·4 m-moles/kg), dissolved in distilled water, by gavage 23 hr before the animal was killed. Food was withheld during this period, but water was available *ad lib*. The effect of cyanide was studied by addition of 0·5 or 1 mM KCN *in vitro* to microsomal preparations.

Pyrazole pretreatment resulted in 26–31 per cent inhibition of the NADPH-dependent MEOS activity, but 89 per cent inhibition of hepatic microsomal catalase activity (Table 1). A similar discrepancy was found in the effects of KCN on the two enzyme systems, a substantial amount of MEOS activity remaining at concentrations of KCN which virtually abolished catalase activity.

In separate experiments (Fig. 1), when NADPH was replaced by the HX/XO system, the degree of inhibition of ethanol oxidation by pyrazole pretreatment and by addition of KCN *in vitro* depended on the concentration of XO. At a concentration of 0·01 unit/ml, the control preparations oxidized ethanol at the same rate as the MEOS, and pyrazole and KCN gave essentially the same degrees of inhibition of the two activities. However, as the XO concentration was increased to 0·02, 0·5 and 1·0 unit/ml the activity in the control preparations rose sharply, while that of the inhibited preparations remained unchanged (Fig. 1). As a result, the per cent inhibition rose steadily with the increasing XO concentration.

TABLE 1. EFFECTS OF PYRAZOLE AND OF KCN ON CATALASE ACTIVITY AND ETHANOL OXIDATION BY RAT LIVER MICROSOMAL PREPARATIONS *in vitro**

Treatment	Catalase		Ethanol oxidation†			
			MEOS		HX/XO system	
	Activity‡	% Inhibition	Activity	% Decrease	Activity§	% Decrease
Control	1·231 ± 0·030 (4,14)		19·99 ± 0·74 (7,14)		101·61 ± 1·60 (13,26)	
Pyrazole	0·129 ± 0·009 (2,8)	89·5	14·36 ± 0·18 (5,10)	28·2	16·79 ± 0·32 (13,26)	84·4
KCN (0·05 mM)	0·074 ± 0·009 (2,6)	94·0	7·54 ± 1·21 (2,4)	62·3	9·23 ± 1·35 (2,4)	81·3
KCN (1·00 mM)	0·022 ± 0·004 (2,6)	98·2	2·43 ± 0·59 (2,4)	87·8	2·00 ± 0·44 (2,4)	95·9

* Values shown are means ±S.E.M. In parentheses is shown the number of separate microsomal preparations per group followed by the total number of replicate determinations.

‡ Catalase activity is expressed as milliequivalents of perborate destroyed per milligram of microsomal protein per 5 min.

† Ethanol oxidation is expressed as nanomoles of acetaldehyde produced per milligram of microsomal protein per 5 min.

§ Values in the pyrazole experiment were obtained at 1·0 unit/ml XO, and per cent inhibition is vs. 1·0 unit/ml XO control shown in the table. Values in the KCN experiment were obtained at 0·5 unit/ml XO, and per cent inhibition is vs. a separate 0·5 unit/ml XO control with a mean value of 49·39 ± 3·96 (4,8).

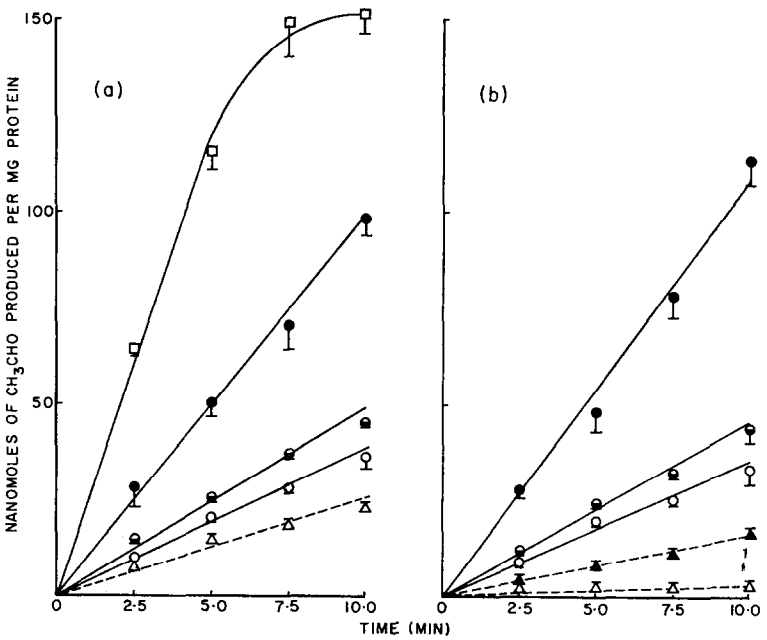

FIG. 1. Production of acetaldehyde from ethanol by rat liver microsomal preparations in the presence and absence of inhibitors. (a) Control values were obtained for peroxidative activity in the presence of different concentrations of xanthine oxidase: ○, 0·01 unit/ml; ◒, 0·02 unit/ml; ●, 0·5 unit/ml and □, 1 unit/ml. MEOS activity supported by NADPH was identical to peroxidative activity at 0·01 unit/ml of xanthine oxidase. Microsomes from pyrazole-pretreated animals gave identical residual activity under all the above conditions, and are shown by the single broken line (△). (b) Control values are shown as in (a). Residual activities in the presence of 0·5 mM (▲) and 1 mM (△) KCN are shown by broken lines. Vertical lines indicate positive or negative standard errors of the means.

The results in Table 1 are in full agreement with those obtained by Lieber and DeCarli[8] under equivalent conditions. Both pyrazole and KCN caused substantially less inhibition of MEOS activity than of microsomal catalase activity or of peroxidative oxidation of ethanol (HX/XO system). However, these findings do not justify the conclusion that MEOS and catalase activities are distinct from each other.

At low concentrations of XO, the low rate of H_2O_2 generation is rate-limiting for catalatic or peroxidative activity, while at high rates of H_2O_2 generation, the activity of catalase itself is the limiting factor. The degree of inhibition produced by any stated dose of pyrazole *in vivo*, or a specified concentration of KCN *in vitro*, depends on the ratio between residual catalase activity and H_2O_2 supply. The behavior of the NADPH-dependent MEOS is indistinguishable, in both the presence and absence of inhibitors, from that of the peroxidative system functioning at a low rate of H_2O_2 generation. Following completion of this work, Thurman *et al.*[11] reported closely similar findings with the use of azide as an inhibitor, and reached the same conclusion.

The differential effects of these inhibitors on the MEOS and catalase activities, under the conditions in which these are normally measured, does not constitute evidence for the existence of a separate microsomal ethanol-oxidizing-system distinct from a combination of NADPH-oxidase plus catalase.

Acknowledgements—This work was supported by the Alcoholism and Drug Addiction Research Foundation of Ontario. We wish to express our gratitude to Dr. Y. Israel for helpful suggestions during the course of this study.

Department of Pharmacology,
University of Toronto,
Toronto 181, *Ontario, Canada*

G. LIN
H. KALANT
J. M. KHANNA

REFERENCES

1. W. H. ORME-JOHNSON and D. M. ZIEGLER, *Biochem. biophys. Res. Commun.* **21,** 78 (1965).
2. C. S. LIEBER and L. M. DECARLI, *J. biol. Chem.* **245,** 2505 (1970).
3. J. M. KHANNA and H. KALANT, *Biochem. Pharmac.* **19,** 2033 (1970).
4. J. M. KHANNA, H. KALANT, G. LIN and G. O. BUSTOS, *Biochem. Pharmac.* **20,** 3269 (1971).
5. M. K. ROACH, W. N. REESE and P. J. CREAVEN, *Biochem. biophys. Res. Commun.* **36,** 596 (1969).
6. J. M. KHANNA, H. KALANT and G. LIN, *Biochem. Pharmac.* **19,** 2493 (1970).
7. E. A. CARTER and K. J. ISSELBACHER, *Ann. N.Y. Acad. Sci.* **179,** 282 (1971).
8. C. S. LIEBER and L. M. DECARLI, *Science, N.Y.* **170,** 78 (1970).
9. H. KALANT, R. K. MURRAY and W. MONS, *Cancer Res.* **24,** 570 (1964).
10. R. N. FEINSTEIN, *J. biol. Chem.* **180,** 1197 (1949).
11. R. G. THURMAN, G. H. LEY and R. SCHOLZ, *Eur. J. Biochem.* **25,** 420 (1972).

Biochemical Pharmacology, Vol. 21, pp. 3308–3312. Pergamon Press, 1972. Printed in Great Britain.

Nucleic acid—Specificity of bleomycin*

(*Received* 14 *April* 1972; *accepted* 8 *August* 1972)

BLEOMYCIN causes a decrease in the melting temperature of DNA and, in addition, produces a scission of DNA strands both *in vitro* and *in vivo*, the former reaction requiring 2-mercaptoethanol or hydrogen peroxide.[1–3] *In vitro*, high concentrations of the drug render about 80 per cent of the DNA trichloroacetic acid (TCA) soluble[4] and also cause the release of free bases from DNA.[5]

In addition to the scission of native DNA strands, Nagai *et al.*[3] showed that bleomycin also caused the fragmentation of the synthetic deoxyribopolymers, polydeoxycytidylate and polydeoxyguanidylate. However, Suzuki *et al.*[6] observed no fragmentation of ribosomal or transfer RNA in the presence of bleomycin.

The present study was initiated to determine the reason for the apparent specificity of bleomycin toward DNA.

The bleomycin was a gift from Bristol Laboratories, Syracuse, N.Y. (lot No. 701233), and from Nippon Kayaku Company, Tokyo, Japan, (lot No. F7071BS). This bleomycin has an absorption maximum at 292 nm. Uridine-2-^{14}C (50·0 mCi/m-mole) was purchased from International Chemical and Nuclear Corp., Irvine, Calif. Polyuridylate-^{3}H (78·1 mCi/m-mole phosphorus) and the copolymer, polydeoxyandenylate-thymidylate-^{3}H (25·1 mCi/m-mole phosphorus) were purchased from Miles Laboratories, Inc., Elkhart, Ind. Uracil-2-^{14}C (56·3 mCi/m-mole) and uridine-5-^{3}H (27·8 Ci/m-mole) were purchased from New England Nuclear, Boston, Mass. Polyadenylate-^{3}H (51·0 mCi/m-mole phosphorus), thymidinemethyl-^{3}H (11·9 Ci/m-mole) and thymine-2-^{14}C (48·6 mCi/m-mole) were purchased from Schwarz BioResearch, Orangeburg, N.Y. Bovine pancreatic ribonuclease A (EC 2.7.7.16), 5 times crystallized, protease-free, was purchased from Sigma Chemical Company, St. Louis, Mo. Yeast ribonucleic acid sodium salt was purchased from Mann Research Laboratories, Inc., New York, N.Y. All other chemicals were purchased from commercial sources.

Labeled bacterial DNA was prepared from *Bacillus subtilis* 168 (*thy trp* C2) as previously described.[4] Uridine-5-^{3}H-labeled DNA, in which deoxyuridylate replaces thymidylate,[6] was prepared from confluent lysis plates of bacteriophage PBS-1 using *B. subtilis* 168 (*trp* C2) as the host bacterium. The plating procedure of Takahashi was used with minor modifications.[7–9]

Labeled RNA was prepared from *B. subtilis* (*thy trp* C2) according to the method of Okamoto *et al.*[10] The bacterial cells were grown as previously described for the isolation of DNA, except that 0·2 μCi/ml of uridine-2 ^{14}C was added to the culture medium.

DNA concentration was assayed by the diphenylamine method of Burton.[11] RNA concentration was assayed by the orcinol method of Hurlbert *et al.*[12]

* This research was supported in part by Grant CA 13246 from the United States Public Health Service and Grant G-441 from the Robert A. Welch Foundation.