Int. J. Biochem. Vol. 22, No. 9, pp. 1005–1008, 1990
Printed in Great Britain.

0020-711X/90 $3.00 + 0.00

HEME REGULATION IN MOUSE MAMMARY CARCINOMA AND LIVER OF TUMOR BEARING MICE—I. EFFECT OF ALLYL-ISOPROPYLACETAMIDE AND VERONAL ON δ-AMINOLEVULINATE SYNTHETASE, CYTOCHROME *P*-450 AND CYTOCHROME OXIDASE*

NORA M. NAVONE, CESAR F. POLO, RODOLFO M. DINGER and ALCIRA M. DEL C. BATLLE†
Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) (CONICET, FCEN, UBA), Ciudad Universitaria, Pabellón 11, 2do Piso, 1428 Buenos Aires, Republica Argentina

(*Received* 18 *January* 1990)

Abstract—1. Basal levels and allyl-isopropylacetamide (AIA) or veronal induced levels of δ-aminolevulinate synthetase (ALA-S), cytochrome *P*-450 (cyt *P*-450) and cytochrome oxidase were determined in tumor (T) and liver of both normal mice (NM) and T bearing mice (TBM).

2. Basal levels of ALA-S were nearly the same in either source. The amount of cyt *P*-450 was lower in TBM liver than in NM liver, and no detectable in T. While the basal activity of cytochrome oxidase in TBM liver and T were higher than those of NM liver.

3. In AIA intoxicated animals there was a lower induction of ALA-S in liver of TBM than in NM liver. There was no induction in T ALA-S. The loss of cyt *P*-450 was less in TBM liver when compared with NM liver.

4. The induction level of cyt *P*-450 after veronal administration was nearly the same in liver of both TBM and NM.

5. We conclude that lower induction of liver ALA-S activity in TBM liver is due to correspondingly lower drug metabolism ability of TBM liver. Otherwise our results suggest that the control mechanism operating in T and probably in its original tissue are different from those described for normal liver.

INTRODUCTION

δ-Aminolevulinate synthetase (ALA-S) (EC 2.3.1.37) is the rate limiting and regulatory enzyme in heme pathway. Administration of porphyrinogenic drugs to animals greatly increased liver ALA-S activity (Granick, 1966).

A number of unsaturated compounds including allyl-isopropylacetamide (AIA) destroy cytochrome *P*-450 (cyt *P*-450), leading to a concomitant acceleration of heme biosynthesis, to restore the levels of hemeprotein (De Matteis, 1970).

On the other hand compounds such as veronal produce a significant decrease in the pool of regulatory free heme by increasing apocyt *P*-450 synthesis. Coordination between the synthesis of the apoprotein and heme has been demonstrated for several hemeproteins such as hemoglobin (Gayzel *et al.*, 1966). So, it has been shown that induction of cyt *P*-450 occurs simultaneously with enhancement in ALA-S activity (De Matteis and Gibbs, 1972). A consistent feature of the chemically induced preneoplastic hepatocyte is their relative resistance to hepatotoxins (Eriksson *et al.*, 1983; Farber *et al.*, 1976). This fact is concordant with metabolic alterations that diminished the capacity of transformed cells to activate xenobiotics to species of high reactiveness (Stout and Becker, 1978) and is also the result of a decreased amount of cyt *P*-450, the terminal acceptor of the monooxygenase system (Oyanagui *et al.*, 1974; Okita *et al.*, 1976; Denk *et al.*, 1980). Stout and Becker (1986) found an increased capacity of heme degradation and a decreased heme synthesis as well as a diminished amount of a number of nonmitochondrial heme proteins in cells arising from chemically induced mouse liver tumors (T). Similar results were observed in both rat liver nodules and carcinoma (Stout and Becker, 1987). Furthermore, Beck *et al.* (1982) have also found and alterated heme pattern in liver from T bearing rats in concordance with findings previously reported by Stout and Becker (1986, 1987).

With the aim of obtaining information as to the regulation mechanism operating in T, liver of T bearing mice (TBM) and liver of normal mice (NM), basal levels and AIA or veronal induced levels of ALA-S, cyt *P*-450 and cytochrome oxidase were determined.

MATERIALS AND METHODS

Animals

Male mice strain BALB/c (20–25 g) were used. Animals were fed Purina 3 diet and given water *ad libitum*. Spontaneous mouse mammary carcinoma from strain BALB/c were used, mice received a 1 mm^3 *innocula* of T injected under skin overlaying the flanks up to the axilla. The animals were killed one month after the implementation. The mice previously heparinized were killed under ether anesthesia by cardiac puncture and bled. In all cases animals were fasted 24 hr before death.

*Dedicated to the memory of Dr Torban K. With.

†To whom correspondence should be addressed at: Viamonte 1881 10A, 1056 Buenos Aires, Republica Argentina.

AIA intoxication

Animals were intoxicated with two s.c. injection of AIA (350 mg/kg body wt) dissolved in 50% ethanol saline, given with an interval of 12 hr. Control mice were injected with the vehicle (50% ethanol saline). Animals were killed 3 hr after the last injection.

Veronal intoxication

Animals were intoxicated with 3 s.c. injection of veronal (167 mg/kg body wt) dissolved in saline solution given every 24 hr. Control mice were injected with saline. Animals were killed 24 hr after the last injection.

Tissue preparation and enzyme assays

ALA-S: Tissues were homogenized (1:3, w/v) in a solution containing: 0.9% NaCl; 0.1 mM Tris–HCl pH 7.4; 0.5 mM EDTA. An aliquot of homogenate was used as enzyme source without previous centrifugation and activity measured as described by Marver *et al.* (1966). An extinction coefficient of $58\,mM^{-1}\ cm^{-1}$ was used to calculate δ-aminolevulinic acid concentration.

Cyt P-450: The microsomal fraction of the homogenates were used and cyt *P*-450 determined by the dithionite-CO absorbance difference, as described by Omura and Sato (1964).

Cytochrome oxidase: The mitochondrial fraction of the homogenates was used and measured according to Yonetani and Ray (1965).

Enzyme units (U) were defined as the amount of enzyme that catalyses the formation of 1 nmol of product under the standard incubation conditions. Specific activity (SA) was expressed as U/mg protein. Protein concentration was determined by the method of Lowry *et al.* (1951).

RESULTS

ALA-S

As shown in Fig. 1 the basal level of ALA-S was nearly the same in either source. In AIA intoxicated animals, liver ALA-S was two-fold increased ($P < 0.05$) in TBM and three-fold increased in NM ($P \ll 0.001$) when compared to controls. The activity of tumoral ALA-S was not induced by AIA. Veronal produced no changes in ALA-S.

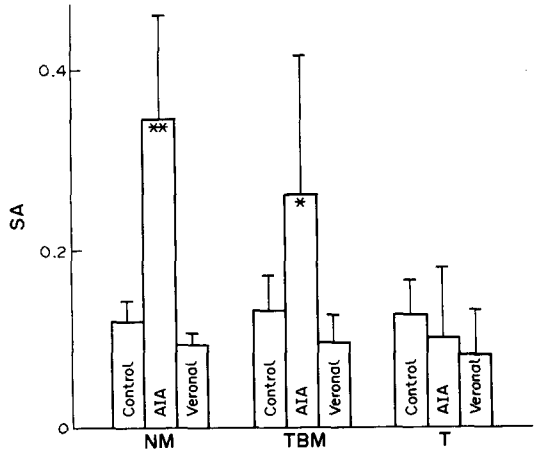

Fig. 1. Levels of ALA-S activity in different tissues of control and AIA and veronal induced animals. Data shown are the average of 6 separate experiments run in triplicates and expressed as means ± SD. $*P < 0.05$; $**P \ll 0.001$. Other experimental conditions were as indicated in the text.

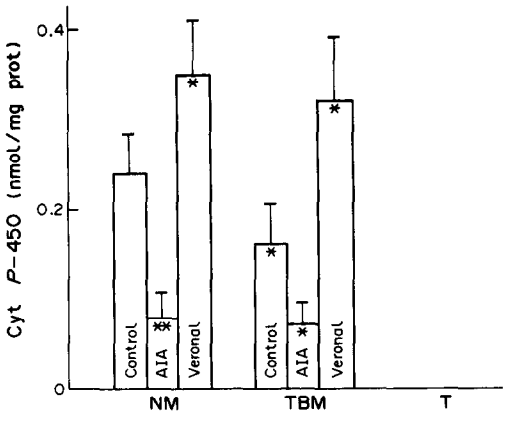

Fig. 2. Levels of cyt *P*-450 in different tissues of control and AIA and veronal induced animals. Data shown are the average of 3 separate experiments run in triplicates and expressed as means ± SD. $*P < 0.05$; $**P < 0.01$. Other experimental conditions were as indicated in the text.

Cyt P-450

Basal levels of liver cyt *P*-450 from TBM were decreased when compared to controls ($P < 0.05$) (Fig. 2). When animals were intoxicated with AIA, liver cyt *P*-450 from NM was 32% ($P < 0.01$) decreased and from TBM 43% ($P < 0.05$) decreased. Veronal instead provoked a significant enhancement of cyt *P*-450 content in liver from both NM and TBM. Tumoral cyt *P*-450 levels were no detectable in any case.

Cytochrome oxidase

Levels of cytochrome oxidase in TBM liver and T were significantly higher ($P < 0.01$) than those of NM liver (Fig. 3). Neither AIA nor veronal produced any variation, in any tissue.

DISCUSSION

Bonkowsky *et al.* (1973) had already reported 16 yr ago that AIA induction of liver ALA-S was lower in T bearing rats than in normal rats, our

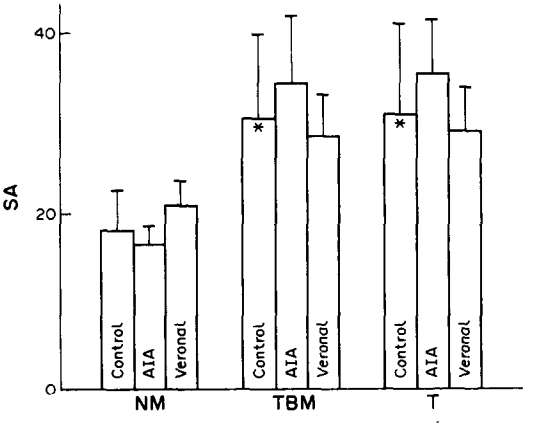

Fig. 3. Levels of cytochrome oxidase activity in different tissues of control and AIA and veronal induced animals. Data shown are the average of 3 separate experiments run in triplicates and expressed as means ± SD. $*P < 0.01$. Other experimental conditions were as indicated in the text.

results are in good agreement with the Americans' findings. But we could now provide some more evidence as to explain the reasons why the levels of ALA-S activity in liver of TBM treated with AIA are lower than in NM. If we take into account that the basal levels of cyt *P*-450 were also lower in TBM liver than in NM liver, that the amount of cyt *P*-450 was nearly equal in liver of both TBM and NM after AIA administration, in other words that the loss of cyt *P*-450 was less in TBM liver and that AIA exerts its porphyrinogenic action only after having been metabolized by cyt *P*-450, it is clear that lower induction of liver ALA-S activity in TBM liver is due to the corresponding lower drug metabolizing ability of TBM liver.

We have also found that veronal did not induce ALA-S, but slightly reduced in either liver from TBM or NM and it did increase cyt *P*-450 content up to nearly the same levels in both TBM and NM liver. Beck *et al.* (1982) have proposed that because of nutritional disorders, and polyamines or hormonal effects occurring in the T, there might be an impairment in the coordinated synthesis of heme and some of those apoproteins having a fast turnover, as a consequence, excess of free heme would be available to both induced heme ozygenase and decreased ALA-S activities, thus explaining the results of these authors but only part of ours. We could justify the observed enhancement of cyt *P*-450, assuming that veronal acts also inducing the synthesis of the cytapoprotein. It is worth taking notice of that when doses >350 mg/kg body wt of veronal were used all TBM died within the second and third day after the first injection, while no NM did, suggesting that the lower initial cyt *P*-450 in TBM liver would diminish the threshold of the veronal lethal dose.

On the other hand basal levels of cytochrome oxidase were higher in TBM liver than in NM liver, this is expected if we consider that there might be an increase of the regulatory free heme pool, according to Beck *et al.* (1982) hypothesis and that free heme mitochondrial fraction which is in equilibrium with that of the regulatory pool, would control cytochrome oxidase levels (Kappas *et al.*, 1983), therefore, enhancement of mitochondrial heme would result in enhancement of cytochrome oxidase.

Regarding now results obtained in T, while ALA-S activity basal levels were the same as those found in liver from either TBM or NM, neither AIA nor veronal produced any changes; more or less the same picture arises for cytochrome oxidase if we compare levels between TBM liver and T and we could not detect cyt *P*-450, even after veronal administration.

These findings are clearly indicating that heme regulation in T should be different from that operating in liver. It has been shown however that T has the same capacity for heme synthesis as it has liver at least up to protoporphyrin formation but it does not seem to produce cyt *P*-450; which is then the fate of the heme produced. It is very likely, therefore, that T makes use of its heme to synthesize heme proteins other than cyt *P*-450, this possibility is supported ty the fact that cytochrome oxidase is greatly increased in T compared with NM liver which emphasizes the hypothesis that the control mechanisms operating in T and probably in its original normal tissue are different from those described for normal liver.

Acknowledgements—This work was supported by grants from the National Research Council (CONICET), University of Buenos Aires and Banco de la Nación Argentina. Alcira M. del C. Batlle holds the post of Principal Scientific Researcher at the CONICET. Nora Navone and César Polo are Research Fellows at the CONICET. Rodolfo Dinger is grateful to Laboratorios PROMECO for a fellowship. We thank Dr N. Andrade and Dr S. Bonaparte from the Intituto Nacional de Oncología "Angel H. Roffo" for providing us the original tumor specimens for transplantation and Joy Ricciardi for some chemicals. We are most grateful to Lic Susana Afonso for the excellent drawings. A. M. del C. Batlle thanks the Ministeriole Educaçion y Ciencia (Spain) and the Association for International Cancer Research (AICR) (U.K.) for special support.

REFERENCES

Beck W. T., Dedmond M. L. and Ouellette T. (1982) Biochemical basis for impaired drug metabolism in tumor bearing rats. *Biochem. Pharmac.* **31,** 1535–1543.

Bonkowsky H., Tschudy D., Collins A. and Doherty J. (1973) Control of δ-aminolevulinic acid synthetase and tyrosine aminotransferase in tumors and livers of tumor-bearing rats. *J. natn. Cancer Inst.* **50,** 1215–1225.

De Matteis F. (1970) Rapid loss of cytochrome P-450 and heme caused in liver microsomes by porphyrinogenic agent 2-allyl-2-isopropylacetamide. *FEBS Lett.* **6,** 343–345.

De Matteis F. and Gibbs A. (1972) Stimulation of liver 5-aminolevulinate synthetase by drugs and its relevance to drug-induced accumulation of cytochrome P-450. *Biochem. J.* **126,** 1149–1160.

Denk H., Abdelfattah-Gad M., Eckerstorfer R. and Talcott R. E. (1980) Microsomal mixed-function oxidase and activities of related enzymes in hyperplastic induced by long-term griseofulvin administration in mouse liver. *Cancer Res.* **40,** 2568–2573.

Eriksson L., Ahluwalia M., Spiewak J., Lee G., Sarma D. S. R., Roomi N. W. and Farber E. (1983) Distinctive biochemical pattern associated with resistance of hepatocytes in hepatocyte nodules during liver carcinogenesis. *Envir. Health Perspect.* **49,** 171–174.

Farber E., Parker S. and Gruenstein M. (1976) The resistance of putative premalignant liver cell populations hyperplastic nodules to the acute cytotoxic effect of some hepatocarcinogens. *Cancer Res.* **36,** 3879–3887.

Gayzel A. I., Hoerchner P. and London I. M. (1966) The stimulation of globin synthesis by heme. *Proc. natn. Acad. Sci. U.S.A.* **55,** 650–655.

Granick S. (1966) The induction *in vitro* of synthesis of δ-aminolevulinic acid synthetase in chemical porphyria: a response to certain drugs, sex hormones and foreign chemicals. *J. biol. Chem.* **241,** 1359–1375.

Kappas A., Sassa S. and Anderson K. E. (1983) The porphyrias. In *The Metabolis Basis of the Inherited Disease* (Edited by Stanbury J., Wyngaarden J., Fredrickson D., Goldstein J. and Brown M.), pp. 1301–1384. McGraw–Hill, New York.

Lowry O., Rosebrough N., Farr A. and Randall R. (1951) Protein measurement with the Folin–Phenol reagent. *J. biol. Chem.* **193,** 265–275.

Marver H., Tschudy D., Perlroth M. and Collins A. (1966) δ-aminolevulinic acid synthetase. I. Studies in liver homogenates. *J. biol. Chem.* **241,** 2803–2809.

Okita K., Noda K., Fujimoto Y. and Takemoto T. (1976) Cytochrome P-450 in hyperplastic nodules during hepatocarcinogenesis with N-2-fluorenylacetamide in rats. *Gann* **67,** 899–902.

Omura T. and Sato R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidences for its hemeprotein nature. *J. biol. Chem.* **239,** 2370–2378.

Oyanagui Y., Sato N. and Hogihara B. (1974) Spectrophotometric analysis of cytochromes in rat liver during carcinogenesis. *Cancer Res.* **34,** 458–462.

Stout D. L. and Becker F. F. (1978) Alterations of the ability of liver microsomes to activate N-2-fluorenylacetamide to a mutagen of *Salmonella typhimurium* during hepatocarcinogenesis. *Cancer Res.* **38,** 2274–2278.

Stout D. L. and Becker F. F. (1986) Heme enzyme patterns in genetically and chemically induced mouse liver tumors. *Cancer Res.* **46,** 2756–2759.

Stout D. L. and Becker F. F. (1987) Heme enzyme patterns in rat liver nodules and tumors. *Cancer Res.* **47,** 963–966.

Yonetani T. and Ray G. (1965) Studies on cytochrome oxidase. VI. Kinetics of the aerobic oxidation of ferrocytochrome c by cytochrome oxidase. *J. biol. Chem.* **240,** 3392–3398.