resting potential did not recover; the rate was (approx.) 12 beats/min, the spikes showing a normal height.

Discussion and summary. In these experiments with atria there was no support for the attractive idea[1] that pyruvate reverses the action of fluorocitrate in causing accumulation of citrate; but it should be stressed that their observations were upon the whole heart. The failure to reverse is, however, consistent with earlier experiments (Peters,[5]). It appears, however, as if there can be some recovery by washing after a low dose of fluoroacetate, which is enough to restore a return of the beat and improved inotropism. This may be due to washing out citrate. Nevertheless, the general toxicity of fluoroacetate could account in part for the asthenic state observed by Peters and Morselli.[6]

Regarding the interesting results with noradrenaline, these may be due to reactivation of phosphokinase on the lines described by Murad *et al.*[7]

Acknowledgements—Our thanks are due to Professor S. Garattini for suggesting the problem and for facilities. We are also grateful to Mrs M. Shorthouse for estimations of citrate. One of us (R.A.P.) thanks the Wellcome Trust for grants in aid of the work in Cambridge.

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Biochemical Pharmacology, Vol. 18, pp 2267–2271. Pergamon Press. 1969. Printed in Great Britain

Effect of cysteine on 5-aminolaevulinate hydrolyase from liver in two cases of experimental intoxication

(*Received* 16 *December* 1968; *accepted* 22 *April* 1969)

THE PRESENT investigation was undertaken to study the effect of -SH groups of cysteine on the activity of 5-aminolaevulinate hydrolyase (δALA dehydratase) (adding 5-aminolaevulinate and cyclizing, EC 4.2.1.24) extracted from the liver of animals receiving chemical compounds known to disturb the porphyrin metabolic pathway.

The ability to induce experimental porphyria by allylisopropylacetamide (AIA) in rats and rabbits has been fully demonstrated.[1–3] In most cases the *in vitro* assay of δALA dehydratase activity in rat liver was carried out using -SH compounds (glutathione, cysteine, etc.).

There have been numerous reports on abnormal levels of porphyrins and porphyrin precursors in animals exhibiting lead poisoning.[4–7]

Granick *et al.* [8] suggested that the direct effect of lead ions on δALA dehydratase might be caused by the inactivation of -SH groups. Dressel and Falk[9] demonstrated an inhibition of haem and porphyrin synthesis *in vitro* from glycine and δALA, respectively, working with lead acetate (10^{-4}M).

The present paper shows the *in vitro* response of the activity of δALA dehydratase to -SH groups in the liver of fasted normal rats in comparison to that of fasted rats treated with AIA. A similar study was carried out in the liver of normal rabbits and rabbits with acute lead poisoning.

The free -SH groups, δALA and protein content in rat and rabbit liver were also measured.

Materials and methods

AIA was kindly provided by Hoffman-La Roche and Co. (Basle); δALA-HCL, *N*-ethylmaleimide (NEMI) and cysteine hydrochloride (Cy) were purchased from Sigma Chemical Co. (St. Louis, Mo.) Dowex 1- × 2-200-400 mesh and Amberlite ICR-50 resins used for the separation of δALA from liver homogenates before measurements[10] were from Dow Chemical Co. and Fisher Scientific Co., respectively.

Animals. Female Wistar rats (weight 90–115 g) were treated according to Marver *et al.*[2] Two subcutaneous (s.c.) injections of AIA (400 mg/kg) at 24 hr intervals were given. Starting 24 hr before the first dose of AIA, food was withheld until sacrifice (18–24 hr after the last dose of AIA). Fasted control animals were given only two s.c. injections of saline solution (NaCl, 8·5 g/l.). After killing the animals by decapitation, their livers were immediately frozen in liquid air and worked up.

New Zealand rabbits weighing 1·1–1·3 kg received a standard laboratory diet, with the addition of fresh vegetables. Acute lead poisoning was induced by the daily s.c. injection of a solution of lead acetate (20 mg of Pb^{++}/ml, pH 5·5) with a dosage range of 20–40 mg as Pb^{++}/kg of body weight per day during 8 days and sacrificed 4 hr after the last injection. The livers of the control animals, receiving the same diet, were worked up. Urine from rats and rabbits gave a positive reaction for porphobilinogen (PBG) with the Ehrlich's reaction.[10] Animals were sacrificed by decapitation and their livers immediately frozen in liquid air.

Enzymic extracts from liver were prepared according to Gibson *et al.*[11] with slight modifications: 1g of tissue was homogenized in a Potter–Elvehjem homogenizer with 3 ml of 0·15 M phosphate buffer (pH 6·9). After centrifugation at 16,500 rev/min (18,000 *g*) for 10 min, the supernatant fraction was used as enzyme source.

Incubation system. 0·3 ml of extract representing 75 mg of tissue, 0·5 ml of 0·15 M phosphate buffer, pH 6·9 (34 μmoles), 1·5 μmoles of δALA-HCl at pH 7 for rat tissue or 0·75 μmole for rabbit liver, 20 μmoles of Cy-HCl, pH 7 (prepared immediately before use when preincubation, 15 min, as indicated) and distilled water up to 1 ml were added to a Thunberg tube. The substrate was placed in the lateral arm when preincubations with Cy were performed. Incubations in anaerobiosis lasted 1 hr at 37°.

Colorimetric measurements. After deproteinizing the incubation mixtures with 30% trichloroacetic acid, aliquots of the filtrate representing 10 mg of tissue were added with warm NEMI solution (50 μmoles) according to Barreiro[12] and heated in a water bath at 55°– 60° for 15 min. After cooling Ehrlich's reaction was performed. Readings were made at 555 mμ. The estimation of δALA was made on deproteinized liver homogenates according to Urata and Granick.[12]

Free -SH groups in liver homogenates were estimated as described by Grunert and Phillips[13] for rat liver and expressed as μmoles of Cy per g of liver; saturated $(NH_4)_2SO_4$ solution instead of NaCl was used in the case of rabbit liver. Protein content in liver homogenates was determined according to Lowry *et al.*[14]

Results

The results obtained working with rat liver are given in Table 1. Experiments No. 1 and 2 represent studies performed on livers from fed rats of the same body weight as the treated animals The values obtained with fasted rats, which did not receive AIA, are given in experiments Nos. 3–6. The figures corresponding to experiments Nos. 7–13 show the results obtained with fasted rats treated with the drug as described before. According to the results, the activity of δALA dehydratase *in vitro* with no preincubation with Cy varied, giving smaller values for fasted normal rats than for fed normal rats. At the same time, livers from rats which received AIA gave lower results than those from normal fasted rats. The most remarkable observation was the *ratio* of optical densities representing activities

TABLE 1. RAT LIVER. EFFECT OF AIA ON SEVERAL BIOCHEMICAL PARAMETERS

Expt. No.	δALA dehydratase activity with no Cy (1)	δALA dehydratase activity with 2×10^{-2} M Cy (2)	2 :1 Ratio	mg protein/ ml homogenate	δALA (mμmoles/g)	Free -SH groups as Cy (μmoles/g)
Fed normal rats						
1	0·22	0·23	1·05	23	—	3·4
2	0·25	0·25	1·00	24	—	2·7
Fasted normal rats						
3	0·16	0·23	1·31	20	26	2·2
4	0·11	0·23	2·09	20	—	1·2
5	0·20	0·27	1·35	21	35	1·7
6	0·21	0·26	1·24	21	32	2·5
		Average value	1·50			
Fasted treated rats						
7	0·08	0·17	2·12	18	12	2·5
8	0·04	0·13	3·25	—	49	—
9	0·10	0·35	3·50	24	30	3·0
10	0·08	0·18	2·50	21	56	1·7
11	0·13	0·29	2·23	21	20	2·2
12	0·15	0·28	1·87	21	48	2·2
13	0·10	0·33	3·30	24	54	2·7
		Average value	2·68			

For explanation see text.

obtained between assays with and without Cy preincubation (see Table 1, column 3). It would seem that δALA dehydratase from fasted rat liver was more activated by the Cy treatment than that from fed rat liver; but the difference is even more striking when comparing the ratios from intoxicated and normal livers.

With respect to δALA content in normal and treated fasted rat livers, higher values were obtained for the latter ones only in few cases.

The average value of free —SH content expressed as Cy would seem slightly higher in experiments

TABLE 2. RABBIT LIVER. EFFECT OF $Pb(CH_3COO)_2$ TREATMENT ON SEVERAL BIOCHEMICAL PARAMETERS

Expt No.	δALA dehydratase activity with no Cy (1)	δALA dehydratase activity with 2×10^{-2} M Cy (2)	2:1 Ratio	mg protein/ ml homogenate	δALA (mμmoles/g)	Free -SH groups as Cy (μmoles/g)
Normal rabbit						
1	0·41	0·41	1·00	32	20	2·1
2	0·42	0·44	1·05	30	33	1·8
3	0·42	0·53	1·26	33	40	2·3
		Average value	1·10			
Treated rabbit						
4	0·24	0·65	2·71	38	45	3·7
5	0·25	0·69	2·76	36	50	4·3
6	0·26	0·68	2·61	38	48	3·7
		Average value	2·69			

For explanation see text.

Nos. 7–13 (average value 2·4 μmoles/g of liver) with respect to that of fasted rats (average value 1·9 μmole/g of liver).

Table 2 represents the values obtained working with both normal rabbit liver and with acute lead poisoning. Experiments Nos. 1–3 represent the values obtained working with fed normal rat liver and Nos. 4–6 those with intoxicated animals. The first column shows δALA dehydratase activity (expressed as optical densities at 555 mμ) obtained with no Cy preincubation. Lower values were observed in the livers of animals treated with Pb^{++}. The second column gives δALA dehydratase activity obtained after preincubation of the enzyme with 2×10^{-2} M Cy for 15 min. The third column represents optical density ratios between values of liver δALA dehydratase activities with and without Cy activation. While the average value for normal livers is 1·10, figures for livers of intoxicated animals showed an increased average value (2·69), representing a great activation induced by Cy in the latter. Similar results were reported by Lichtman and Feldman[15] about the effect of glutathione working on erythrocytes both of normal patients and of those with lead poisoning.

δALA contents, in spite of the reported lower activity of δALA synthetase,[6] are higher in treated than in normal rabbit livers. The same result was obtained for free -SH group contents.

Discussion

Many and very different drugs have shown an ability to induce disturbances on the tetrapyrrol pathway.[16] Efforts to demonstrate the significance of chemical structure in this effect have been made by several authors.[17,18] Granick[16] has demonstrated the induction which several compounds (steroids among them) exert on δALA synthetase activity, the first enzyme of this metabolic chain.

Levere[19] demonstrated an increase in δALA synthetase activity in hepatic cirrhosis and postulated that an inability for conjugating sex steroids would arise from a defect in the uridyl diphosphate glucuronyl transferase, leaving free unconjugated steroids to act as inducers. The activity of the last enzyme appears very diminished in porphyric rat liver.[20] In spite of Levere's postulation, Hayashi *et al.*[21] were able to demonstrate that the AIA-induced increase of δALA synthetase was strongly inhibited by bilirrubine (a competitive compound of some steroids for conjugating glucuronic acid); injection of bilirrubine also reduced the enzyme level in control rats which had not received AIA.

The sulphydrillic character of δALA synthetase has not yet been fully demonstrated. However, although Cy inhibited its activity probably by combining pyridoxal phosphate,[22] an essential co-factor for its action, arsenite (which would provide As^{+++}), and $CdSO_4$ (ref. 2), *p*-chloromercuri-benzoate and Pb^{++} (ref. 9), markedly reduce the synthesis of δALA.

The numerous papers published in relation to δALA dehydratase from several biological sources have allowed it to be included among sulphydryl enzymes.[11,24,25] Although the mechanism of AIA action on δALA synthetase and its metabolic fate is not yet clearly understood, the other compound, $Pb(CH_3COO)_2$ tested in this work probably acts by blocking -SH groups coming both from the enzyme or from metabolites; these chemical combinations are expected to be irreversible. However, results indicated a marked increased δALA dehydratase activity with Cy preincubations. It is also interesting to stand out the higher content of free -SH groups found in acute lead poisoned liver than that found in the normal one.

If one considers that the main effective porphyriogenic compounds, AIA, Sedormid, diallyl-barbiturate and others, all possess ketonic groups able to enolize and to form hemiketals with free -SH groups, it would be interesting to study the level of enzymes related to the production of -SH compounds, such as glutathione dehydroascorbate oxidoreductase, under the effect of those drugs. Such a study would be necessary in order to explain the higher values of free -SH groups in lead poisoning.

The fact that Lichtman and Feldman[15] found enhancement in activation by reduced glutathione on δALA dehydratase in erythrocytes belonging to patients with plumbism, with respect to normal cases, reinforces the significance of free -SH concentration in regulating the enzyme activity. Also Greenberg[22] observed the high content of glutathione in erythrocytes, noting the possibility of a control mechanism of enzymes through their -SH groups; should this control exist it might have been affected in case of intoxication with lead or other drugs which can react with -SH groups.

The similar response to -SH group activation of δALA dehydratase from liver in the two cases of intoxication here presented would lead to postulate, at least, a common action of the two compounds on the free -SH groups coming from both the enzyme or free -SH group containing metabolites.

Acknowledgements—The author wishes to express her gratitude to Dr. Luis F. Leloir and Dr. Carlos E. Cardini for allowing the use of the laboratories of the Instituto de Investigaciones Bioquimicas for the performance of this work.

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Biochemical Pharmacology, Vol. 18, pp 2271–2274. Pergamon Press. 1969. Printed in Great Britain

Effect of propyl gallate on carbon tetrachloride induced fatty liver

(*Received* 30 *December* 1968; *accepted* 22 *April* 1969)

IT HAS been suggested that carbon tetrachloride-induced fatty liver is mediated by the stimulated lipid peroxidation.[1–6] Hepatic fat infiltration has been found to be both partially prevented by tocopherol[7] and inhibited by compounds other than antioxidants.[8] These findings do not seem to fit with the hypothesis that lipid peroxidation plays the most important role in the pathogenesis of the CCl_4-induced fatty liver. Propyl gallate is a water soluble antioxidant which has been shown to