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CARDIAC δ-AMINOLEVULINIC ACID SYNTHETASE ACTIVITY

EFFECTS OF FASTING, COBALTOUS CHLORIDE AND HEMIN

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Abstract—Cardiac δ-aminolevulinic acid (ALA) synthetase activity was studied in animals that had been fasted. The kinetics of the decline in cardiac ALA synthetase activity were measured for 48 hr after food was withdrawn from rats. A marked rate of decrease in activity was observed between 12 and 24 hr. Cardiac ornithine decarboxylase activity also decreased in fasted rats, but cardiac tyrosine aminotransferase activity remained unchanged. Cardiac ALA synthetase activity also decreased in fasted mice but did not decrease significantly in fasted guinea pigs or fasted rabbits whose activites were low even in the fed state. Cobaltous chloride administration caused a marked decrease in cardiac ALA synthetase activity which remained low for a longer duration of time than has been observed in rat liver. The administration of hemin, which causes a marked decrease in hepatic ALA synthetase activity, had no effect on cardiac ALA synthetase activity. The difference in the responses of hepatic and cardiac ALA synthetase activities to hemin administration and food deprivation suggests differential regulation of ALA synthetase activity in heart and liver.

δ-Aminolevulinic acid (ALA) synthetase, the initial enzyme in the heme biosynthetic pathway, is generally considered to be the principal site in the control of the rate of heme synthesis in mammalian and avian liver [1–4]. The physiological regulation of the rate of heme synthesis by endogenous substances occurs primarily by altering ALA synthetase activity, and heme itself may regulate the rate of its own synthesis by either repressing the synthesis of ALA synthetase [5, 6] or by directly inhibiting the activity of the enzyme [7, 8]. Hormones and their metabolites cause marked changes in ALA synthetase activity in developing avian liver [2, 9, 10], and may also have a role in maintaining [11] and inducing [12–14] ALA synthetase activity in rat liver. In addition to alterations in ALA synthetase activity caused by endogenous substances, many xenobiotics have marked effects on hepatic ALA synthetase activity. The administration of phenobarbital, 3,5-dicarbethoxy-1,4-dihydrocollidine, or allylisopropylacetamide causes a marked increase in hepatic ALA synthetase activity [15, 16], while cobaltous chloride administration causes a marked decrease in hepatic ALA synthetase activity [17–20].

Although one expects the heart to require synthesis of heme to maintain many of its normal metabolic functions, almost nothing is known about the process of heme synthesis in the heart. The probable rate-controlling enzyme of cardiac heme synthesis, ALA synthetase, has been detected by Israels *et al.* [21] in chick embryo heart and by Briggs *et al.* [22] and Abraham and Terjung [23] in rat heart. Observations made by Briggs *et al.* [22] suggest that cardiac ALA synthetase activity may be regulated differently from hepatic ALA synthetase activity. Fasting of rats for 24 and 48 hr caused a marked decrease in cardiac ALA synthetase activity but had little effect on hepatic ALA synthetase activity. Allylisopropylacetamide and 3,5-dicarbethoxy-1,4-dihydrocollidine, potent inducers of hepatic ALA synthetase activity, also had no effect on the activity of the cardiac enzyme [22].

The current investigations were designed to pursue the regulation of cardiac ALA synthetase activity. They describe (a) the kinetics of decrease of cardiac ALA synthetase activity in the fasting rat and in other species, (b) decreases in the activities of other cardiac enzymes in fasted rats, and (c) the effects of glucose, hemin and cobaltous chloride administration on cardiac ALA synthetase activity.

MATERIALS AND METHODS

Pyridoxal phosphate, GTP, coenzyme A (free acid), succinic thiokinase, hemin (type III), dithiothreitol, ornithine monochloride, tyrosine and α-ketoglutarate were obtained from the Sigma Chemical Co., St. Louis, MO. The sodium form of AG 50 WX8, 200–400 mesh, was obtained from BioRad Laboratories, Richmond, CA. [2,3-^{14}C]Succinic acid (45 mCi/mmole) and DL-[1-^{14}C]ornithine monohydrochloride (45 mCi/mmole) were purchased from New England Nuclear, Boston, MA. Dexamethasone sodium phosphate injection (Hexadrol) was obtained from Organon, West Orange, NJ.

Male albino rabbits (2 kg), male albino mice (30 g), male guinea pigs (450 g) or male Sprague–Dawley rats (180–250 g) were used. Animals were allowed food and water *ad lib.* except when food was withheld for an indicated duration of time. When used, glucose (6.7 g/kg) was administered by oral intubation at 18, 15 and 12 hr prior to death [24]. All other compounds were administered subcutaneously in the

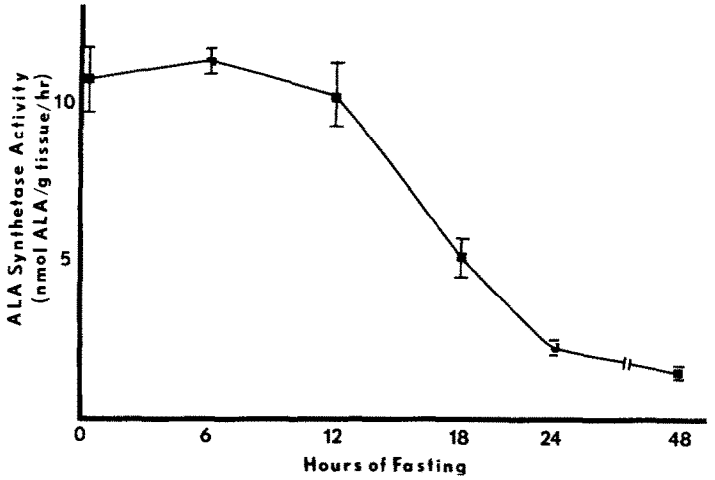

Fig. 1. Decline of cardiac ALA synthetase activity with time in the fasting rat. Food was withdrawn at the beginning of a 12 hr light cycle. ALA synthetase activity was determined by the method of Briggs *et al.* [22]. Each point represents the mean of at least five animals.

following manner: Alloxan (200 mg/kg), 7 days prior to death [25]; glucagon (5.0 mg/kg), 12 hr prior to death [26]; dexamethasone (100 μg/rat). twice daily for 2 days prior to death [22]; hemin (20 mg/kg, dissolved in 0.1 N NaOH, pH adjusted to 7.4) at the time period indicated prior to death; cobaltous chloride·$6H_2O$ (60 mg/kg) at the time periods indicated prior to death.

Hepatic ALA synthetase activity was determined by the method of Ebert *et al.* [27]. Cardiac ALA synthetase activity was determined in homogenate and mitochondrial preparations using modifications of the method of Briggs *et al.* [22]. Incubation was terminated by the addition of 1 ml of 10% trichloroacetic acid instead of 25% trichloroacetic acid [22], and the protein precipitates were then washed with 3 ml of 2% trichloroacetic acid. Three milliliters of 0.2 M sodium acetate solution were then added to the pooled acid extracts. No carrier ALA was added to the samples prior to their placement on ion exchange columns. EDTA was deleted from the reaction mixture when mitochondrial preparations were assayed. In studies where hemin was added to mitochondrial preparations, the hemin was dissolved in 0.1 N NaOH and the pH was adjusted to 7.6 with Tris–HCl buffer.

Cardiac tyrosine aminotransferase activity was determined by the method of Granner and Tomkins [28]. Cardiac ornithine decarboxylase activity was determined by the following modifications of the method of Matsushita *et al.* [29]. The reaction mixture containing 0.2 mM ornithine, a concentration which was determined to be optimal for cardiac tissue, and 0.5 ml of 2 M sodium hydroxide was placed in the center well of a Warburg flask to collect $^{14}CO_2$. Radioactivity was determined by a method described previously [30].

Cardiac tissue was prepared for analysis of cobalt by atomic absorption spectroscopy analysis in the following manner. The tissue (0.5–0.7 g) was homogenized with a minimum of distilled water, utilizing a polytron homogenizer. The homogenate was digested with 2 ml of concentrated nitric acid at 50–60° for 24 hr. After cooling, an additional 2 ml of nitric acid were added. The samples were heated gently until solubilized, and then 2 ml of 30% hydrogen peroxide were added and the samples were again heated gently until bubbling subsided. The samples were then diluted to 5.0 ml and analyzed by a Perkin–Elmer model 360 atomic absorption spectrophotometer using an air–acetylene flame at the 240.7 nm line of a cobalt hollow cathode lamp.

Blood glucose was determined by the method of Thompson [31]. Protein was determined by the method of Lowry *et al.* [32]. Statistical evaluations were made using Student's *t*-test.

RESULTS

Although studies from our laboratory have shown that cardiac ALA synthetase activity is reduced markedly in rats fasted for 24 or 48 hr [22], the rate of decline of cardiac ALA synthetase activity in rats

Table 1. Cardiac ALA synthetase activity in fed and fasted rat, guinea pig, mouse and rabbit

Species	Cardiac ALA synthetase activity* (nmoles/g heart/hr) Fed	Fasted†
Rat	17.3 ± 1.0 (12)	4.4 ± 0.5‡ (12)
Mouse	20.6 ± 2.0 (5)	6.0 ± 0.5‡ (5)
Guinea pig	4.8 ± 0.5 (5)	4.0 ± 0.5 (5)
Rabbit	5.9 ± 1.0 (5)	3.8 ± 0.5 (5)

* Values represent group mean ± standard error. The number of animals per group is given in parentheses.

† Rats, mice and rabbits were fasted for 24 hr; guinea pigs were fasted for 48 hr.

‡ Denotes significant decrease from fed animals ($P < 0.05$).

Table 2. Effects of dexamethasone on ornithine decarboxylase activity and tyrosine aminotransferase activity in the rat heart

Treatment	Ornithine decarboxylase activity* (nmoles/g heart/hr)	Tyrosine aminotransferase activity* (μmoles/g heart/hr)
Fed	10.0 ± 1.0 (5)	7.3 ± 0.2 (6)
Fed + dexamethasone†	3.5 ± 0.5‡ (5)	8.6 ± 0.7 (5)
24-hr Fast	3.0 ± 0.5‡ (5)	8.8 ± 0.2‡ (5)
24-hr Fast + dexamethasone†	3.0 ± 0.05 (5)	9.3 ± 0.4 (5)

* Values represent the group mean ± standard error. The number of animals per group is given in parentheses.
† Dexamethasone (100 μg/rat) was administered subcutaneously twice daily for 2 days.
‡ Denotes significant difference from fed control values ($P < 0.05$).

following food withdrawal has not been determined. These data are shown in Fig. 1. Following the withdrawal of food, cardiac ALA synthetase activity remained fairly constant for about 12 hr, after which time a marked and rapid decrease of ALA synthetase activity occurred. The level of ALA synthetase activity in fasted rats fell eventually to about 20 per cent of the activity detected in rats which were allowed food *ad lib*. This magnitude of decrease has been noted previously [22].

Several other species were studied in order to determine whether the decline of cardiac ALA synthetase activity was characteristic only of the rat, or whether it could be observed in other species. Cardiac ALA synthetase activity was decreased substantially in mice fasted for 24 hr (Table 1) but in fasted guinea pigs or rabbits decreases in activity were not statistically significant. However, it should be noted that only a low level of activity was detected in the hearts from these animals when they were allowed food *ad lib.*, and the variability might have obscured real decreases in ALA synthetase activity.

In order to determine whether fasting caused a selective decrease of cardiac ALA synthetase activity or a generalized decrease in the activities of other cardiac enzymes, ornithine decarboxylase activity and tyrosine aminotransferase activity were determined both in rats allowed food and in rats fasted for 24 hr. These enzymes were chosen because they were considered to be enzymes which turn over rapidly in liver [33]. Fasting caused a marked decline in cardiac ornithine decarboxylase activity (Table 2) (in agreement with the result reported by Krelhaus *et al.* [34]), but caused a significant increase of cardiac tyrosine aminotransferase activity.

Briggs *et al.* [22] showed that the administration of dexamethasone prevented the decrease of cardiac ALA synthetase activity in fasted rats. The administration of dexamethasone at a dose which prevented the decline of cardiac ALA synthetase in fasting rats caused a marked decrease of cardiac ornithine decarboxylase activity in fed rats and had no effect on the activity of the enzyme in fasted rats (Table 2). Dexamethasone administration had no significant effect on cardiac tyrosine aminotransferase activity.

Studies by Tschudy *et al.* [35] and Bonkowsky *et al.* [24] have shown that carbohydrate administration prevents induction of hepatic ALA synthetase activity by allylisopropylacetamide in rats. The administration of a dose of glucose which prevented the induction of hepatic ALA synthetase activity caused only a slight increase in cardiac ALA synthetase activity in both fed and fasted rats (data not shown). Also, two substances which alter glucose metabolism in the rat, alloxan and glucagon, were administered to determine their effects on cardiac ALA synthetase activity. Although blood glucose levels rose to a level of 500 mg/100 ml after alloxan administration, only a slight decrease of cardiac ALA synthetase activity was observed. Glucagon administration had no effect on cardiac ALA synthetase activity. Thus, neither glucose feeding nor modification of insulin or glucagon levels appears to be involved in mediating the decline of ALA synthetase activity which occurs during fasting.

Table 3. Cardiac ALA synthetase activity following cobaltous chloride administration in the rat

Treatment	Time after treatment (hr)	ALA synthetase activity* (nmoles/g heart/hr)
Fed rat		13.5 ± 2.5
Cobalt†	1	7.5 ± 1.0‡
Cobalt†	4	6.0 ± 0.5‡
Cobalt†	12	6.0 ± 0.5‡

* Values represent group mean ± standard error of five rats.
† $CoCl_2 \cdot 6H_2O$ was administered to fed animals at a dose of 60 mg/kg subcutaneously.
‡ Significantly different from fed rats ($P < 0.05$).

Table 4. Effect of cobaltous chloride on cardiac ALA synthetase activity *in vitro*

Treatment	ALA synthetase activity* (nmole/mg protein/hr)
Fed rat	0.29
24-hr Fasted rat	0.07
Cobalt† (0.05 mM)	0.30
Cobalt† (0.1 mM)	0.27
Cobalt† (0.5 mM)	0.17

* Each value represents the mean of two experiments.
† Incubations contained mitochondria prepared from hearts of fed rats. ALA synthetase activity was determined by the method of Briggs *et al.* [22] except that EDTA was omitted from reaction mixtures.

Table 5. Effects of hemin administration on cardiac and hepatic ALA synthetase activity in the rat

Treatment	Time after treatment (hr)	ALA synthetase activity* (nmoles/g/hr) Liver	Heart
Fed rat		24.0 ± 2.5	12.5 ± 1.0
24-hr Fasted rat			3.0 ± 0.5‡
Hemin†	3	11.5 ± 2.5‡	16.0 ± 2.0
Hemin†	6	3.5 ± 0.5‡	13.0 ± 1.5
Hemin†	12	13.0 ± 2.0‡	13.5 ± 2.5
Hemin†	24	24.0 ± 3.5	13.0 ± 1.5

* Values represent group mean ± standard error of at least four rats.
† Hemin was administered to fed rats at a dose of 20 mg/kg subcutaneously.
‡ Denotes statistically significant decrease compared with fed rats ($P < 0.05$).

Cobaltous chloride administration *in vivo* causes a marked decline in hepatic ALA synthetase activity [17–20]. The effect of cobaltous chloride administration to rats on cardiac ALA synthetase activity is shown in Table 3. Treatment of rats allowed food *ad lib.* caused a marked decrease in cardiac ALA synthetase activity 1 hr after administration, and the level of cardiac ALA synthetase activity remained low for at least 12 hr after treatment. At times later than 12 hr, assessment of ALA synthetase activity was complicated by the fact that the rats did not eat, and a combination of the effects of cobaltous chloride administration and fasting prevented an appropriate analysis of the mechanism of decrease of ALA synthetase activity. The level of cobalt in the heart (± S.E.), determined at 30 and 60 min following cobalt administration, was 56 ± 7 nmoles/g heart and 64 ± 6 nmoles/g heart respectively. Cobalt had no direct inhibitory effect on cardiac ALA synthetase *in vitro* (Table 4) at concentrations in the same order of magnitude as those detected in the heart *in vivo*.

Hemin administration to rats also causes decreases in hepatic ALA synthetase activity [36]. While hemin treatment caused a substantial decrease of hepatic ALA synthetase activity at 3, 6 and 12 hr after administration, cardiac ALA synthetase activity remained at control levels after hemin treatment (Table 5). However, cardiac ALA synthetase activity was inhibited by hemin *in vitro* in mitochondrial preparations (Table 6), indicating that hemin has the potential of inhibiting ALA synthetase activity and may regulate by directly inhibiting ALA synthetase activity under certain conditions.

Table 6. Effect of hemin on cardiac ALA synthetase activity *in vitro*

Treatment	ALA synthetase activity* (nmole/mg protein/hr)
Fed rat	0.43
24-hr Fasted rat	0.10
Hemin† (25 μM)	0.35
Hemin† (50 μM)	0.31
Hemin† (100 μM)	0.23

* Each value represents the mean of two experiments.
† Incubations contained mitochondria prepared from hearts of fed rats. ALA synthetase activity was determined by the method of Briggs *et al.* [22].

DISCUSSION

The current studies show that there is a rapid decline in cardiac ALA synthetase activity in rats and mice when food is withdrawn for relatively short periods of time. Marked decreases in cardiac ornithine decarboxylase activity were also observed in fasted rats, in confirmation of the results of Krelhaus *et al.* [34]. Gertz and Haugaard [37] have observed decreases in cardiac uridine kinase activity in fasted rats. However, the activities of all cardiac enzymes do not decrease in the fasting state. Cardiac tyrosine aminotransferase activity is increased in fasting rats and, in studies employing fasted mice or rabbits, Wildenthal *et al.* [38] observed no differences or slight increases in the activities of cathepsin D, acid phosphatase and β-acetylglucoamidase. ALA synthetase, ornithine decarboxylase and uridine kinase are thought to be rate-limiting steps in biosynthetic processes, while tyrosine aminotransferase, cathepsin D, acid phosphatase and β-acetylglucosamidase are enzymes involved in catabolic processes. Thus, it may be that during fasting the heart preserves enzymes involved in catabolic processes, while allowing reduction of activities of enzymes involved in biosynthetic processes.

The administration of a dose of dexamethasone which prevents a decrease of ALA synthetase activity in fasting rats [22] failed to prevent a decrease of cardiac ornithine decarboxylase activity in fasted rats and caused a marked decrease in cardiac ornithine decarboxylase activity in the fed rat. Thus, even though both cardiac ALA synthetase and cardiac ornithine decarboxylase activities decline in the fasting state, the different responses of these enzymes to dexamethasone treatment indicate independent regulation of these enzymes in the heart.

The decrease of ALA synthetase activity during fasting is also characteristic of mice, but is apparently not characteristic of guinea pigs or rabbits. These latter two species possessed low levels of cardiac ALA synthetase activity in both the fed and fasted state. Irving and Elliott [39] detected very low levels of ALA synthetase activity in mitochondria from

guinea pig liver. They also reported the existence of an inhibitory substance in guinea pig liver mitochondria which inhibited ALA synthetase activity in mitochondria obtained from 3,5-dicarbethoxy-1,4-dihydrocollidine-treated animals. The low levels of ALA synthetase activity detected in guinea pig and rabbit hearts could have been caused by the presence of an endogenous inhibitory substance present in the hearts of these species. The decrease of cardiac ALA synthetase activity in fasted rats does not appear to be caused by the presence of an endogenous inhibitor. Studies were performed where mixtures of cardiac homogenates from fed and fasted rats were analyzed for ALA synthetase activity. The ALA synthetase activities obtained in mixtures of cardiac homogenates from fed and fasted rats were additive.

It is well established that hepatic ALA synthetase activity is reduced markedly following cobaltous chloride administration [17–20]. This report documents a marked decrease in cardiac ALA synthetase activity following cobaltous chloride administration. The decrease in ALA synthetase activity in the heart following cobaltous chloride administration is maintained for a duration of time longer than that observed in the liver. It is well known that the heart is a primary site of cobalt toxicity [40–43], and our results may be a sign of relatively selective effects of cobalt on that organ. Cardiac ALA synthetase activity was not inhibited directly by cobalt *in vitro*, a characteristic it shares with the hepatic enzyme. The mechanism of the rapid decline of cardiac ALA synthetase activity following cobalt administration cannot be defined as yet, but recently two reports have indicated that cobalt protoporphyrin IX may be generated *in vivo*, and may be the factor involved in inhibiting ALA synthetase activity [44–45].

The observation that fasting causes a marked decrease in cardiac ALA synthetase activity but has no effect on hepatic ALA synthetase activity suggests that ALA synthetase is regulated differently in the rat heart and rat liver. Differences in the regulation of these enzymes are also suggested by the responses of the cardiac and hepatic enzymes to hemin administration. While hemin administration caused a decrease in hepatic ALA synthetase activity, it had no effect on the cardiac enzyme. The difference in the response of cardiac and hepatic ALA synthetase activity to hemin administration could be due simply to the inability of exogenously administered heme to enter cardiac cells, it is also possible that the synthesis of cardiac ALA synthetase may not be repressed by hemin, as has been postulated to occur for hepatic ALA synthetase activity [2].

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