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EFFECTS OF PORPHYRINOGENIC (PORPHYRIA-PRODUCING) AGENTS ON AVIAN EMBRYONIC HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY

Tin-Chuen Yeung[1] and Anthony S. Gidari[2]

Department of Pharmacology
State University of New York
Downstate Medical Center
Brooklyn, NY 11203

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SUMMARY: Embryonic avian hepatic glutathione S-transferase activity was not significantly altered after 17-day-old chick embryos or cultured embryonic hepatocytes were treated for 24 h with various agents that influence δ-aminolevulinic acid (ALA) synthase activity. 2-allyl-2-isopropylacetamide, an enlarger of ALA synthase, increased hepatic heme and cytochrome P-450 levels without altering the GSH S-transferase activity during 24 h of drug treatment. With a longer treatment schedule (3 to 4 days), however, GSH S-transferase activity was induced by 2-allyl-2-isopropylacetamide. Thus, the early induction of ALA synthase was dissociated from the induction of GSH S-transferase activity and does not appear to be an indirect effect mediated by a decrease in free heme as a result of the induction of heme-binding GSH S-transferases.

Hepatic heme biosynthesis is regulated by negative feedback repression (1) of heme on δ-aminolevulinic acid (ALA[3]) synthase (EC 2.3.1.37), the first and the rate-limiting enzyme in hepatic heme synthesis (2,3). Although the precise mechanism underlying this repression has not been established, it appears that the ALA synthase level is directly or indirectly regulated, at some posttranscriptional step (1), by free intracellular heme levels (4,5). It is likely that this free heme pool is exchangeable with heme bound to hemoproteins, since exogenously administered heme is able to bind to pre-existing apohemoproteins such as apotryptophan pyrrolase (6,7) and apocyto-

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1. Present address: Department of Pharmacology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

2. To whom reprints should be addressed at: SUNY, Downstate Medical Center, 450 Clarkson Avenue (Box 29), Brooklyn, NY 11203.

3. Abbreviations used are: ALA, δ-aminolevulinic acid; AIA, 2-allyl-2-isopropylacetamide; GST, GSH S-transferases; PIA, 2-propyl-2-isopropyl-acetamide; PB, phenobarbital; DNCB, 1-chloro-2,4-dinitrobenzene.

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chrome P-450 (8-10) to reconstitute the functional enzymes. Although much attention has been focused on these hemoproteins as sites of "regulatory" heme, another class of liver proteins, the GSH S-transferases (GST; EC 2.5.1.18), has also been shown to bind heme noncovalently (11). This suggests that GSH S-transferases may be an alternate site of the putative "regulatory" heme pool. In addition, hepatic GSH S-transferase activity is inducible by many xenobiotics (12). Two of these agents, 2-allyl-2-isopropylacetamide (AIA) and phenobarbital (PB) are also inducers of ALA synthase. Thus, the induction of ALA synthase by AIA or PB may be due, in part, to an increase in GSH S-transferase levels, which, by binding free intracellular "regulatory" heme, may reduce the size of the repressive "regulatory" heme pool. In order to investigate this hypothesis, we studied the effects of porphyrinogenic (porphyria-producing) agents on embryonic chicken liver GSH S-transferase activity to determine if these agents induce GSH S-transferase activity under conditions in which ALA synthase is induced. We have previously purified a species of adult chicken liver GSH S-transferase and have shown that this protein, like its rat hepatic counterpart, binds heme with a dissociation constant of 2.2×10^{-8} M (13).

MATERIALS AND METHODS

1,2-Dichloro-4-nitrobenzene (DNCB) was purchased from Aldrich Chem. Co. (Milwaukee, WI). GSH and hemin were obtained from Sigma Chem. Co. (St.Louis, MO). AIA and 2-propyl-2-isopropylacetamide (PIA) were from Hoffman-LaRoche Inc. (Nutley, NJ). Cyproterone was a gift from Schering AG (Berlin). Phenobarbital was a product of Merck and Co. (Rahway, NJ).

GSH S-transferase assays were performed according to the method of Habig *et al.* (14) as previously described (13). DNCB was used as the substrate as indicated. One unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 μmole of GSH-drug conjugate per min.

Cytochrome P-450 in the whole liver homogenate was determined from dithionite-reduced CO *vs.* CO difference spectra in a Beckman Model UV5230 split-beam spectrophotometer as described by McLean and Day (15). Heme was measured by the pyridine-hemochromogen method (16).

In ovo experiments were performed with fertilized White Leghorn chicken (*Gallus domesticus*) eggs obtained from Shamrock Farms, NJ. Test chemicals in appropriate vehicles (0.2 to 0.4 ml) were injected through a hole made in the shell over the air sacs. Eggs were incubated at 37° in a humidified incubator

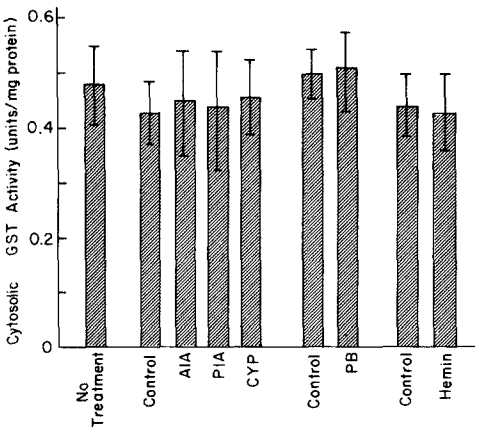

Fig. 1. Effects of various agents on embryonic chicken hepatic GSH S-transferases. Chick embryos (17-day-old) were injected with various agents: ALA, 2 mg/embryo (in propylene glycol); PIA, 2 mg/embryo (in propylene glycol); cyproterone, 2 mg/embryo (in propylene glycol); PB, 2 mg/embryo (in 0.9% NaCl); hemin, 0.4 ml of a 1 mM solution in ethanol/10 mM NaOH (1:1, v/v). Controls received only the corresponding vehicles. Cytosolic GSH S-transferase activity was measured after 24 h with DNCB as the substrate. Each value represents the mean of 4 to 6 experiments.

gased with 5% CO_2 for the designated length of time. Livers were perfused through the heart with 10 ml ice-cold 0.9% NaCl prior to removal. Livers from 3 chick embryos were pooled and homogenized in 0.05 M Tris (pH 7.4) containing 0.25 M sucrose. Cytosol was prepared as the 100,000 x g supernatant (for 90 min) of the homogenate.

Embryonic chicken liver cells from 17-day-old embryos were also cultured as described (17). After the addition of various test agents, the cultures were allowed to incubate for various lengths of time as indicated for each experiment. The cells were collected and homogenized in 100 mM potassium phosphate (pH 6.7). GSH S-transferase activity was measured in the cytosolic fraction, which was the supernatant obtained after centrifugating the cell homogenate at 100,000 x g for 90 min.

Protein was determined by the method of Lowry et al. (18).

RESULTS AND DISCUSSION

Several agents which are known to influence heme biosynthesis were tested to determine whether they had any effect on the chick embryonic GSH S-transferase activity. Of these agents, AIA, PB, PIA (19) and cyproterone (17) are inducers of embryonic chick liver ALA synthase. In addition, AIA and PB are inducers of rat liver GSH S-transferase (12). No significant change in the hepatic GSH S-transferase activity was detected after treatment of the 17-day-old embryos (Fig. 1) or cultured embryonic liver cells (Table I) with

Table I. Effects of various agents on cytosolic GSH S-transferase activity in primary chick liver cell culture.

Treatment	Cytosolic GSH S-Transferase Activity
	units/mg protein
Control	0.36 ± 0.04
AIA (100 µg/ml)	0.32 ± 0.03
Cyproterone (20 µg/ml)	0.30 ± 0.03
Phenobarbital (25 µg/ml)	0.31 ± 0.04
Hemin (20 µg/ml)	0.34 ± 0.01

Cells were cultured from 17-day-old chick embryonic liver as described in "Methods." Cytosolic GSH S-transferase activity was measured 24 h after treatment with various agents in ethanol (final concentration of ethanol was 0.1%) except hemin, which was dissolved in ethanol/10 mM NaOH (1:1, v/v).

the various agents for 24 h. Under these conditions and at the same concentrations, these agents have been shown to either induce or, in the case of hemin, to inhibit the induction of ALA synthase (17,19).

GSH S-transferase activity could be detected in the culture medium after culturing the cells for 48 h. However, no difference in the enzymatic activity was detected whether the cells had been previously treated with AIA or not (data not shown). This indicates that the inability to detect an AIA-mediated difference in cytosolic GSH S-transferase levels 24 h after drug treatment was not due to a difference in the rate of extrusion of the enzyme from the drug-treated cultured cells.

The relationship between avian hepatic GSH S-transferase levels and the amounts of total hepatic heme and cytochrome P-450 after administration of AIA was also studied during the first 24 h of drug treatment in ovo (Fig. 2). The amount of cytochrome P-450 measured in the homogenate increased about 3-fold at the end of the 24 h. Total heme in the homogenate was only slightly elevated. Cytosolic GSH S-transferase remained virtually unchanged during the whole 24-hour period.

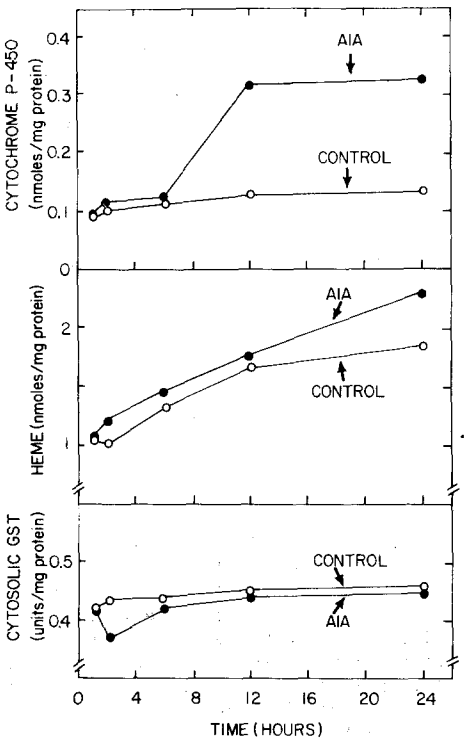

Fig. 2. Effects of AIA on embryonic liver homogenate cytochrome P-450, heme, and cytosolic GSH S-transferase. Chick embryos (17-day-old) were injected with 2 mg/embryo of AIA in 0.2 ml propylene glycol. Controls received only 0.2 ml propylene glycol.

The long-term effects of AIA on avian hepatic GSH S-transferase activities *in ovo* and in culture were studied in order to determine whether a long drug treatment schedule is required to induce the transferases. AIA treatment of 16-day-old embryos for 4 days or cultured embryonic liver cells for 3 days significantly increased the cytosolic GSH S-transferase level by 100% and 30%, respectively (Table II). This indicates that the embryonic avian hepatic GSH S-transferases, like their rat counterparts, are inducible by AIA. The long period of drug treatment needed for induction also suggests that avian GSH S-transferases have a long half-life, since proteins with short half-lives are subject to rapid increases or decreases by alterations in their synthesis and/or degradation (20). Rat liver ligandin (GSH S-transferase B) has a relatively long half-life of 2.3 days (21), and the

Table II. Effects of long-term treatment with AIA on avian GSH S-transferase activity *in ovo* and *in vitro*.

	Cytosolic GSH S-Transferase Activity
	units/mg protein
1. *In ovo*	
Control (n = 6)	0.49 ± 0.04
AIA treatment (n = 6)	1.04 ± 0.04*
2. *In vitro*	
Control (n = 6)	0.71 ± 0.04
AIA treatment (n = 6)	1.04 ± 0.14*

Chick embryos (16-day-old) received an initial dose of 2 mg/egg AIA in propylene glycol for the first day, followed by a daily dose of 1 mg/embryo AIA for the next three days. Embryos were sacrificed 24 h after the last dose of AIA. Controls received only propylene glycol. Embryonic chicken hepatocytes were cultured from 17-day-old embryonic chicken liver. AIA (100 μg/ml) was added to the hepatocytes after culturing the cells for 24 h, and cytosolic GSH S-transferase was assayed with DNCB as the substrate after 72 h of drug treatment.

*$p < 0.05$ *vs.* corresponding controls.

induction of rat liver GSH S-transferases by several drugs required a long treatment schedule of 7 to 10 days (12,22-25).

The present results indicate the AIA and other porphyrinogenic agents did not significantly alter embryonic chicken liver GSH S-transferase activity under conditions in which ALA synthase is induced, although GSH S-transferase activity is inducible, however, both *in ovo* and in culture, by a longer treatment schedule with AIA. Additionally, treatment of 17-day-old chick embryos with AIA for 24 h significantly increased hepatic heme and cytochrome P-450 without changing the GSH S-transferase levels. Thus, the induction of ALA synthase by AIA and, presumably, other porphyrinogenic compounds, is not an indirect effect mediated by the induction of the heme-binding GSH S-transferases.

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