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Effects of Interferon Inducing Agents (Polyriboinosinic Acid·Polyribocytidylic Acid, Tilorone) on Hepatic Hemoproteins (Cytochrome P-450, Catalase, Tryptophan 2,3-Dioxygenase, Mitochondrial Cytochromes), Heme Metabolism and Cytochrome P-450-Linked Monooxygenase Systems

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SUMMARY

EL AZHARY, ROKEA & MANNERING, GILBERT J. (1979) Effects of interferon inducing agents (polyriboinosinic acid·polyribocytidylic acid, tilorone) on hepatic hemoproteins (cytochrome P-450, catalase, tryptophan 2,3-dioxygenase, mitochondrial cytochromes), heme metabolism and cytochrome P-450-linked monooxygenase systems. *Mol. Pharmacol.* **15**, 698–707.

Single doses of the interferon inducing agents, poly rI·rC and tilorone, were shown to depress hepatic cytochrome P-450, cytochrome b_5, catalase and tryptophan 2,3-dioxygenase. The cytochrome P-450 level of microsomes declined progressively after a single dose of poly rI·rC for 40 hr and then recovered to within 25% of the control level by 96 hr. The ethylmorphine N-demethylase activity of the microsomes paralleled the cytochrome P-450 level except during the first 4 hr after administration of poly rI·rC when there was a greater loss of demethylase activity than could be accounted for by the loss of cytochrome P-450. Tilorone had effects similar to those of poly rI·rC on cytochrome P-450 and ethylmorphine N-demethylase activity, but its action was delayed. Temporal aspects of the loss and recovery of the catalase of liver homogenates after the administration of poly rI·rC or tilorone were similar to those of microsomal cytochrome P-450. About 60% of the total tryptophan 2,3-dioxygenase (apo + holo enzyme) activity of the 100,000 × *g* supernatant fraction of liver homogenate was lost within 6 hr of the administration of poly rI·rC; activity returned to the control level by 15 hr. However, there was a transient rise in the ratio of holo:total tryptophan 2,3-dioxygenase activity at 3 hr which returned to the control value by 5 hr. Tilorone had no statistically significant effect on either the total activity of this enzyme or the ratio of holo to total enzyme. The increase in the ratio of holo:total enzyme was followed by a depression of δ-aminolevulinic acid synthetase (ALA-S) activity at 5 hr and an induction of heme oxygenase activity at 6 to 8 hr. ALA-S activity returned to the control level within 24 hr, but the induced level of heme oxygenase activity remained for 40 hr and did not return to normal until 96 hr. Tilorone had a similar but delayed effect on ALA-S activity. Tilorone induced heme oxygenase activity to a degree equal to that produced by poly rI·rC, but a much longer time was required to reach maximal activity (2 to 3 hr versus 20 to 30 hr). These temporal aspects of the effects of interferon inducing agents on the ratio of holo:total tryptophan 2,3-dioxygenase, ALA-S and heme oxygenase are thought to reflect changes in the size of an

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unassigned heme pool that regulates the synthesis of heme. Mitochondrial cytochromes a, b, and c were not depressed significantly after 4 daily doses of poly rI·rC; cytochrome c_1 was depressed about 20%. The cytochrome P-450 content and benzo[a]pyrene activity of intestinal mucosa and adrenal were not affected by 4 daily doses of poly rI·rC; the cytochrome P-450 content of the kidney cortex was not altered, but the benzo[a]pyrene hydroxylase activity was lowered by 40%.

INTRODUCTION

In two previous publications from this laboratory (1, 2) we showed that a wide variety of interferon-inducing agents depress hepatic cytochrome P-450-linked monooxygenase systems and concluded that the depression of these systems is a general property of interferon-inducing agents. Induction of cytochrome P-450 by phenobarbital or benzo[a]pyrene is associated with an increase in ALA-S[2] activity (1). Several investigators (3–7) have postulated that the induction of ALA-S is a response to a decrease in the size of an unassigned regulatory heme pool. The decrease in ALA-S activity produced by the administration of endotoxin may occur as a result of an increase in the size of the heme pool (6). Heme oxygenase, the rate-limiting enzyme in heme degradation, destroys excess heme and thereby contributes to the function of the heme pool. Apotryptophan 2,3 dioxygenase combines with heme to form tryptophan 2,3-dioxygenase (holoenzyme), the active form of the enzyme. Changes in the amount of the holoenzymes relative to the apoenzyme are thought to reflect alterations in the size of the heme pool (7).

Endotoxin is a potent interferon-inducing agent (8). If endotoxin depresses cytochrome P-450-linked monooxygenase systems because it is an interferon-inducing agent, then other interferon-inducing agents should also depress ALA-S and induce heme oxygenase. Studies were undertaken to test this possibility and the feasibility of the involvement of a regulatory heme pool. The current communication reports on temporal aspects of the effects of the interferon-inducing agents poly rI·rC and tilorone, on microsomal cytochrome P-450, ALA-S, heme oxygenase and monooxygenase activities and on the degree of heme saturation of tryptophan 2,3-dioxygenase (holoenzyme/total enzyme).

The possibility was considered that hepatic hemoproteins other than cytochrome P-450 might be depressed by interferon-inducing agents. Accordingly, the effects of administered tilorone and poly rI·rC on levels of hepatic catalase, tryptophan 2,3-dioxygenase (apoenzyme + holoenzyme) and mitochondrial cytochromes were determined. The effects of these interferon-inducing agents on certain extrahepatic cytochrome P-450 systems were also studied.

MATERIALS AND METHODS

Materials. Poly rI·rC, δ-aminolevulinic acid, $NADP^+$, glucose-6-phosphate, NADPH, hemin (equine), Dowex X-100 resin (200–400 mesh), pyridoxal phosphate, L-tryptophan, sodium ascorbate, methemoglobin and sodium deoxycholate were purchased from Sigma Chemical Co., St. Louis, Missouri. Ethylmorphine HCl was purchased from Mallinckrodt Chemical Works, St. Louis, Missouri. Succinic acid-2,3-^{14} was purchased from New England Nuclear Corp., Boston, Massachusetts. Glucose-6-phosphate dehydrogenase was obtained from Boehringer Mannheim Corp., New York, N.Y. Benzo[*a*]pyrene was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. Tilorone hydrochloride was a gift from Richardson-Merrell, Inc., Cincinnati, Ohio.

Treatment of animals. Male Sprague-Dawley rats (150–200 g) from Biolab Company were fed Purina Rat Chow *ad libitum* until killed. Control rats received 0.5 ml of 0.9% saline (i.p.), or 1 ml of saline (p.o.). Poly rI·rC in 0.9% saline was injected (i.p.) in a single dose of 10 mg/kg except when its effects on hepatic mitochondrial cytochromes and the benzo[*a*]pyrene hydroxylase activities of extrahepatic tissues were

[2] The abbreviations used are: ALA-S, δ-aminolevulinic acid synthetase; poly rI·rC, polyriboinosinic acid·polyribocytidylic acid.

measured, in which case it was administered for 4 days rather than one day. Tilorone·HCl in 0.9% saline was given in a single dose of 50 mg/kg (p.o.). Animals were killed between 7:00 and 10:00 a.m.

Tissue preparations. ALA-S and catalase activities of liver homogenates were determined. For ALA-S determinations, 0.25% homogenates in NaCl-tris buffer (0.01 M, pH 7.4) were used. For catalase determination, 10% homogenate in NaCl 0.01 M phosphate buffer (pH 7.4) were centrifuged at 600 × *g* for 10 min; diluted supernate was used for the determination. Levels of cytochromes P-450 and b_5 and monooxygenase and heme oxygenase activities were determined in microsomes prepared as described previously (9). The 100,000 × *g* supernate obtained from the preparation of microsomes was used for the determination of tryptophan 2,3-dioxygenase activity. Hepatic mitochondria were prepared as described by Johnson and Lardy (10). Intestinal microsomes and adrenal homogenates were prepared as described previously (11). Renal cortex microsomes were prepared in the same manner as hepatic microsomes.

Assays. ALA-S activities of liver homogenates were determined by the radiometric method of Ebert *et al.* (12) except that a 0.25 rather than a 1.0% homogenate was used.

Heme oxygenase activity of microsomes was determined by the method of Tenhunen *et al.* (13). Tryptophan 2,3-dioxygenase is present in liver cytosol in the active holoenzyme (heme saturated) and inactive apoenzyme forms. Total enzyme (holo + apo) was measured by saturating the apoenzyme with heme to form holoenzyme as described by Knox *et al.* (14).

Catalase activity of liver homogenates, prepared as described by Cohen *et al.* (15), was determined by the spectrophotometric method of Beers and Sizer (16) as described by Nakamura *et al.* (17). Hydrogen peroxide was assigned an extinction coefficient of 0.0436 $mM^{-1}\ cm^{-1}$ at 240 nm. One unit of catalase decomposes one micromole of H_2O_2/min at 25°.

The spectrophotometric method of Williams (18) was used to determine mitochondrial cytochromes a, b, c_1 and c.

Microsomal ethylmorphine N-demethylase activity was determined as described previously (9). Benzo[a]pyrene hydroxylase activity was determined by the method of Wattenberg *et al.* (19).

Cytochrome P-450 was assayed by the method of Omura and Sato (20). The method of Strittmatter and Velick (21) was used for the determination of cytochrome b_5.

Statistical analysis. Data were subjected to statistical analysis using Student's *t*-test. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Losses and recoveries of hepatic cytochrome P-450, cytochrome b_5, and ethylmorphine N-demethylase activities after a single dose of poly rI·rC or tilorone. The effects of a single dose of poly rI·rC (10 mg/kg, i.p.) on hepatic cytochrome P-450, cytochromes b_5 and ethylmorphine N-demethylase activity are shown in Fig. 1. At 2 and 4 hr after the injection there was no effect on the microsomal cytochrome P-450 level. By 6 hr, the cytochrome P-450 content was depressed to 84% of controls. Thereafter, the decline was progressive until a maximum loss of 62% was reached at 40 hr. By 96 hr the level had returned to within 75% of control. Ethylmorphine Ndemethylase activity paralleled cytochrome P-450 content except at the 4 hr period, at which time there was a 36% loss of demethylase activity without a measurable loss of cytochrome P-450. Losses of ethylmorphine N-demethylase activity and cytochrome P-450 were about equal after 24 hr. The maximum loss of cytochrome b_5 was 30% and this occurred 40 hr after poly rI·rC administration.

The time of onset of action of a single dose of tilorone (50 mg/kg, p.o.) on the depression of cytochrome P-450 and ethylmorphine N-demethylase activity was later than that of poly rI·rC (Fig. 2). The first significant depression of cytochrome P-450 occurred 16 to 24 hr after tilorone administration; at 24 hr the cytochrome P-450 content of microsomes was 84% of control and ethylmorphine N-demethylase activity was 50% of control. Thus, the depression of

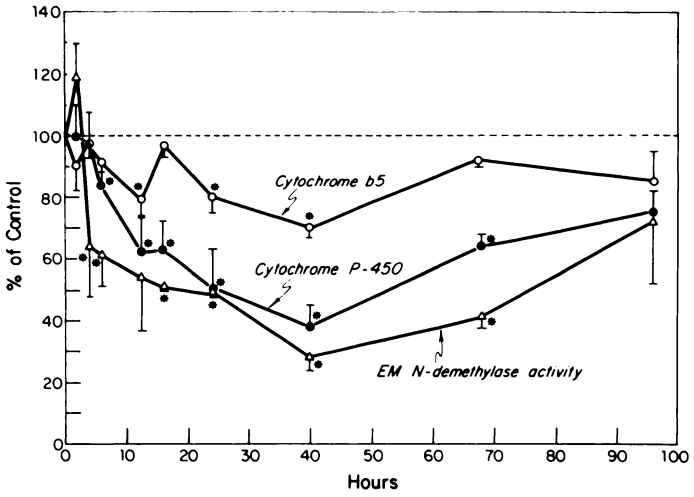

FIG. 1. *Time courses of loss and recovery of cytochrome P-450 and cytochrome b_5 contents and ethylmorphine (EM) N-demethylase activity of hepatic microsomes after a single injection of poly rI·rC (10 mg/kg, i.p.)*

Control rats received 0.5 ml of saline (0.9%) i.p. Animals were killed at indicated times after the injections and microsomes were prepared from their livers. Values are mean percentages ± SE of control values. N = 3 (EM N-demethylase activity) or 6 (cytochromes P-450 and b_5). 100% control values: EM N-demethylase activity, 6.81 ± 0.77 nmole of HCHO formed/mg of protein/min; cytochrome P-450, 0.74 ± 0.05 nmole/mg of protein; cytochrome b_3, 0.38 ± 0.02 nmole/mg of protein. *Significantly different from control value ($p < 0.05$).

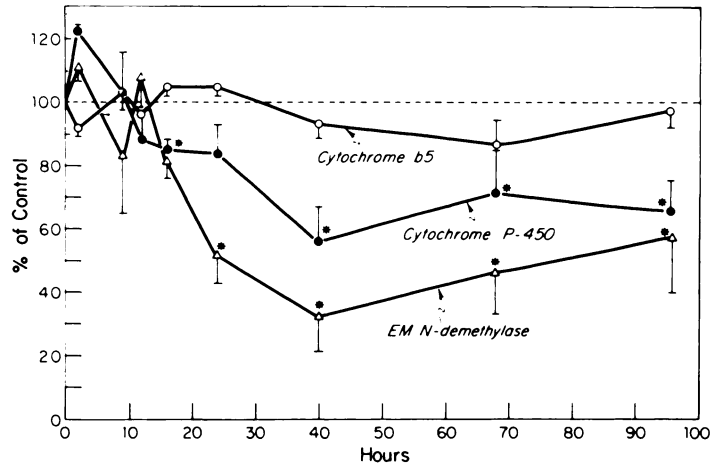

FIG. 2. *Time course of loss and recovery of cytochrome P-450 and cytochrome b_5 contents and ethylmorphine (EM) N-demethylase activity of hepatic microsomes after a single dose of tilorone (50 mg/kg, p.o.).*

Experimental conditions were the same as those given in Fig. 1 except for the substitution of tilorone for poly rI·rC. 100% control values were obtained from the same animals which provided data for Fig. 1. *Significantly different from control value ($p < 0.05$).

demethylase activity both preceded and exceeded the loss of cytochrome P-450 during the first 24 hr of the experiment. Maximum depression was observed at 40 hr, at which time cytochrome P-450 was 55% of control and ethylmorphine N-demethylase activity was 32% of control. By 96 hr, cytochrome P-450 content and demethylase activity were within 70% and 58% of control values, respectively. Cytochrome b_5 was not depressed significantly at any time throughout the experiment.

Loss and recovery of hepatic catalase after a single dose of poly rI·rC or tilorone. The loss and recovery of catalase activity from homogenates of liver after a single dose of poly rI·rC or tilorone are shown in Fig. 3. Catalase activity was depressed to 75% of control 12 hr after poly rI·rC administration. Tilorone had no significant effect at that time. The maximum depression of catalase in poly rI·rC-treated rats occurred between 24–40 hr, at which time catalase activity was 64% of the control value. Recovery was nearly complete at the end of 140 hr. A depression of catalase activity was not observed until 48 hr after the administration of tilorone, at which time there was a 26% loss of activity. A maximum depression of 37% occurred at 96 hr. By 140 hr, catalase activity had returned to 84% of the control value.

Loss and recovery of tryptophan 2,3-dioxygenase after a single dose of poly rI·rC or tilorone. The effects of a single dose of poly rI·rC on tryptophan 2,3-dioxygenase activity (holoenzyme) and total enzyme (holoenzyme + apoenzyme) are shown in Fig. 4. Three hours after poly rI·rC administration there was a 40% rise in holoenzyme activity but no change in the total enzyme activity. By 6 hr, both total enzyme activity and the holoenzyme level were decreased to 58% and 66% of controls, respectively. Levels of the total enzyme had returned to the control level by 15 hr, but the heme saturated enzyme was still depressed by 68%. However, by 24 hr the level had returned to 90% of the control value.

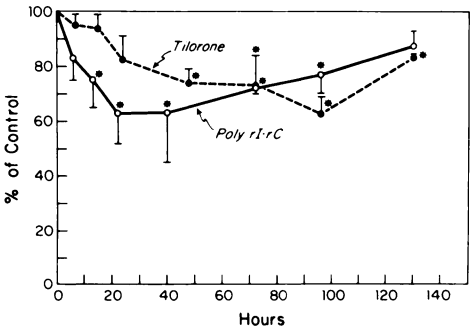

FIG. 3. *Time course of loss and recovery of hepatic catalase after a single dose of poly rI·rC (10 mg/kg, i.p.) or tilorone (50 mg/kg, p.o.)*

Control rats received 0.5 ml of saline (0.9%), i.p., or 1 ml, p.o. Animals were killed at indicated times after the administration of poly rI·rC or tilorone and their livers were homogenized. Values are mean percentages ± SE of control values (N = 4). 100% control catalase activity: 23,825 ± 1867 units/g of liver. *Significantly different from control value ($p < 0.05$).

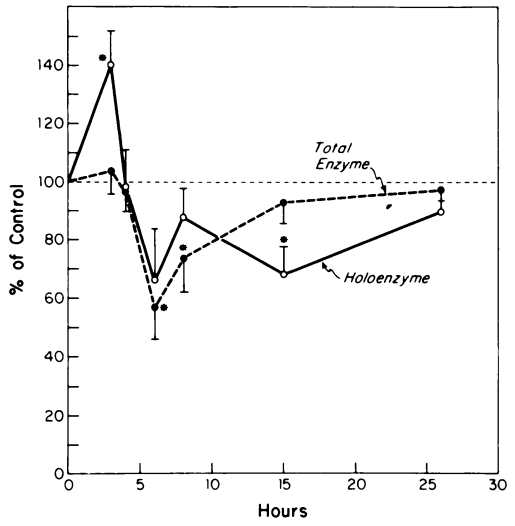

FIG. 4. *Time course of loss and recovery of hepatic tryptophan 2,3-dioxygenase after a single injection of poly rI·rC (10 mg/kg, i.p.).*

Treatment of animals was the same as that given in Fig. 1. Values are mean percentages ± SE of control values (N = 4). 100% control total tryptophan 2,3-dioxygenase activity: 26.2 ± 2.1 nmole kynurenine formed/mg of protein/hr. 100% control holoenzyme activity: 14.4 ± 1.2 nmole kynurenine formed/mg of protein/hr.

Neither total enzyme nor holoenzyme activity were altered appreciably by a single dose (50 mg/kg) of tilorone (data not shown).

Loss and recovery of ALA-S after a single dose of poly rI·rC or tilorone. The time course of loss and recovery of ALA-S after a single dose of poly rI·rC or tilorone is shown in Fig. 5. Three hours after poly rI·rC administration the ALA-S activity of liver homogenate was 80% of that of controls; by 5 hr the activity was depressed maximally to 52% of the control level. The activity returned to control values by 24 hr. No effect of tilorone was observed at 5 hr, but at 10 hr the activity was depressed maximally to 66% of control values. ALA-S activity returned to the control level by 24 hr.

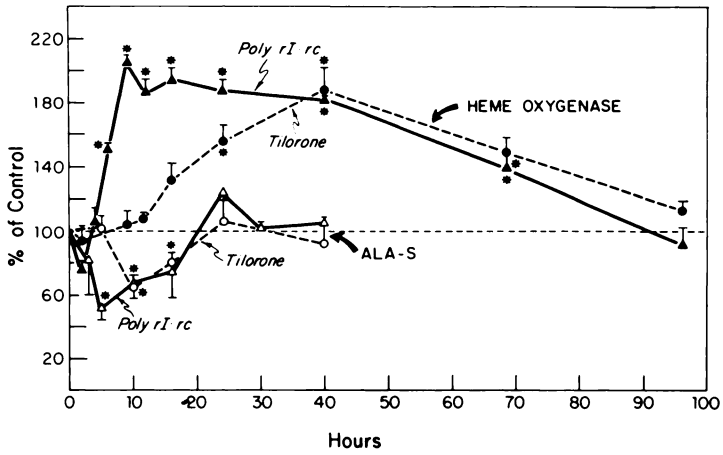

FIG. 5. *Time course of loss and recovery of ALA-S and of induction and recovery of heme oxygenase after a single dose of poly rI·rC or tilorone*

Treatment of animals was the same as that given in Fig. 3. Values are mean percentages ± SE of control values. N = 6 (heme oxygenase), or 4 (ALA-S). 100% control heme oxygenase activity: 0.61 ± .049 nmole bilirubin formed/mg of protein/10 min. 100% control ALA-S activity: 33.7 ± 3.2 nmole ALA-S formed/g of liver/hr.

Induction of heme oxygenase activity by a single dose of poly rI·rC or tilorone. Six hours after poly rI·rC administration microsomal heme oxygenase activity was increased 50% above the control level (Fig. 5). Activity increased progressively until it reached a maximum of 210% of the control value at 8 hr. Activity remained induced for about 30 hr and then declined slowly until control levels were reached by 96 hr.

Tilorone induced heme oxygenase later than poly rI·rC. The first significant increase was observed at 24 hr at which time heme oxygenase activity was 54% above the control level (Fig. 5). A maximum induction of heme oxygenase to 190% of the control value was observed 40 hr after tilorone administration. Control levels were reached by 96 hr.

Effect of multiple doses of poly rI·rC on mitochondrial cytochromes a, b, c_1 and c. The effects of four daily doses of poly rI·rC (10 mg/kg/day) on mitochondrial cytochromes a, b, c_1 and c are summarized in Table 1. Only the loss of cytochrome c_1 (20%) was statistically significant.

Effect of poly rI·rC on extrahepatic cytochrome P-450 and benzo[a]pyrene hydroxylase activity. Poly rI·rC was injected daily for 4 days and cytochrome P-450 content and benzo[a]pyrene hydroxylase activity were determined in the adrenal gland, kidney cortex, intestinal mucosa and liver (Table 2). Adrenal cytochrome P-450 and benzo[a]pyrene hydroxylase activity were not depressed by poly rI·rC. Kidney cortex benzo[a]pyrene hydroxylase activity was depressed to 60% of control values, but the cytochrome P-450 content was not significantly affected. The intestinal cytochrome P-450 level and benzo[a]pyrene hydroxylase activity were not depressed. Hepatic cytochrome P-450 and benzo[a]pyrene hydroxylase activities were depressed by 40 and 70%, respectively, in these animals.

Effects of poly rI·rC and tilorone on hepatic ALA-S, heme oxygenase, catalase and tryptophan 2,3-dioxygenase activities in vitro. Neither poly rI·rC (0.0005 or 0.05 mg/ml) nor tilorone (0.01, 0.1 or 1 mM) had an effect on the ethylmorphine N-demethylase or heme oxygenase activities of microsomes, the ALA-S and catalase activities of homogenates or the tryptophan 2,3-dioxygenase activity of 100,000 × *g* supernate (data not shown).

DISCUSSION

Previous studies from this laboratory demonstrated that interferon-inducing agents depress hepatic cytochrome P-450 (1, 2). The current study shows that the

TABLE 1

Effect of poly rI·rC on hepatic mitochondrial cytochromes a, b, c_1 and c

	Mitochondrial cytochromes (nmole/mg mitochondrial protein)			
	a	b	c_1	c
Control	0.17 ± 0.02 (6)[b]	0.25 ± 0.01 (6)	0.19 ± 0.01 (6)	0.17 ± 0.01 (6)
Poly rI·rC	0.14 ± 0.01 (6)	0.22 ± 0.01 (6)	0.15 ± 0.01[a] (6)	0.15 ± 0.01 (6)
(% of control)	(85%)	(90%)	(80%)	(88%)

Rats received (i.p.) 10 mg poly rI·rC/kg, daily for 4 days. Control rats received 0.5 ml saline (i.p.).
Values represent mean ± SE.
[a] Significantly different from controls ($p < 0.05$).
[b] Values in parentheses = number of animals.

TABLE 2

Effect of poly rI·rC on cytochrome P-450 content and benzo[a]pyrene hydroxylase activity of the adrenal, kidney cortex, intestinal mucosa and liver

	Benzo[a]pyrene hydroxylase (nmole 3-HOBP formed/mg protein/hr		Cytochrome P-450 (nmole/mg protein)	
	Control	Poly rI·rC	Control	Poly rI·rC
Whole adrenal homogenate	0.91 (2)[a]	0.90 (2)	0.08 (2)	0.06 (2)
(% of control)		(99%)		(82%)
Kidney cortex microsomes	0.54 ± 0.07 (5)[b]	0.32 ± 0.03[c] (5)	0.07 ± 0.02 (5)	0.06 ± 0.01 (5)
(% of control)		(60%)		(87%)
Intestinal mucosa homogenate	2.1 ± 0.4 (3)[d]	2.38 ± 0.14 (3)	.01 ± 0.0 (3)	.01 ± 0.0 (3)
(% of control)		(113%)		(98%)
Liver microsomes	25.4 ± 2.2 (4)[e]	7.71 ± 0.75[c] (4)	0.79 ± 0.04 (4)	0.48 ± 0.02[c] (4)
(% of control)		(30%)		(60%)

Rats received 10 mg of poly rI·rC (i.p.)/kg daily for 4 days. Control rats received 0.5 ml saline (i.p.).
Values represent mean ± SE.
[a] Values obtained from 2 pools of 12 adrenals from 6 rats.
[b] Values obtained from 3 pools of 6 kidneys from 3 rats.
[c] Significantly different from controls ($p < 0.05$).
[d] Values obtained from 3 pools of 3 small intestines from 9 rats.
[e] Values obtained from 4 livers.

interferon-inducing agents, poly rI·rC and tilorone, also depress cytochrome b_5 hepatic catalase and tryptophan 2,3-dioxygenase (Figs. 1, 3, 4). These agents also caused a depression of ALA-S, an induction of heme oxygenase and an early rise in the heme saturation of apotryptophan 2,3-dioxygenase. Figure 6 illustrates the temporal order of these events. The first event is a short-lived increase in the conversion of apotryptophan 2,3-dioxygenase to the active heme-saturated holoenzyme. This occurs within 3 hr of the administration of poly rI·rC. At this time ALA-S was partially depressed and heme oxygenase was not affected. At 5 hr the ratio of holo to total tryptophan 2,3-dioxygenase had returned to normal, and the depression of ALA-S was maximal. Induction of heme oxygenase activity began between 4 and 6 hr and reached a maximum at 8 hr. The induced level of heme oxygenase activity was maintained throughout the period during which ALA-S and the ratio of holo to total tryptophan dioxygenase levels were returning to normal. If one accepts the concept that heme synthesis is regulated by a heme pool (3–7), the sequence of these events can be interpreted as follows: a) interferon-inducing agents cause an increase in the size of the regulatory heme pool. This could occur if these agents cause an increased dissociation of heme from cytochrome P-450 or other hepatic hemoproteins. It could also occur if

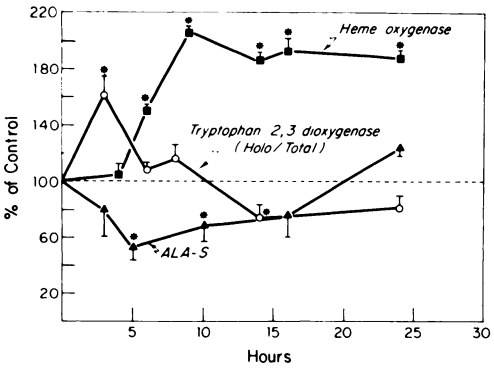

FIG. 6. *Temporal aspects of the effect of a single dose of poly rI·rC (10 mg/kg) on the ALA-S and heme oxygenase activities and the relative heme saturation of tryptophan 2,3-dioxygenase*

The figure was constructed from data presented in Figs. 4 and 5.

the interferon inducing agents depress the synthesis of apocytochrome P-450 and the apoproteins of other hemoproteins, thereby decreasing the demand for heme from the heme pool; b) the temporary increase in the ratio of holo to total tryptophan 2,3-dioxygenase is caused by the temporary increase in the size of the heme pool (7); c) the increase in the size of the heme pool depresses ALA-S synthesis; d) heme in excess of that necessary to depress ALA-S synthesis induces heme oxygenase, which relieves the inhibition of ALA-S by destroying heme.

As shown in Figs. 1, 2 and 4, there are no appreciable losses of cytochrome P-450, cytochrome b_5, catalase or total tryptophan 2,4-dioxygenase during the first 3 hr after the administration of poly rI·rC, yet the loss of ALA-S was appreciable and the increase in holo tryptophan 2,3-dioxygenase was maximal at this time (Figs. 5 and 6). This might raise the question as to how heme that had dissociated from hemoproteins could have increased the size of the heme pool, and thereby triggered the biochemical events that followed. There is about 160 times more cytochrome P-450 in liver than tryptophan 2,3-dioxygenase (22). Thus, if the heme sequestered by apotryptophan 2,3-dioxygenase represents a substantial portion of the heme released from hemoprotein, only a very small amount of cytochrome P-450 would have to dissociate to produce a large increase in the holo enzyme.

The time courses of the depression of the hemoproteins after poly rI·rC or tilorone differed widely. This can be attributed to the turnover rates of the respective enzymes. Under steady state conditions the level of an enzyme is determined by its rate of synthesis and its rate of degradation. An alteration in either rate can affect the enzyme level. However, according to Schimke (23), the time required for an enzyme to change from one steady state level to another is dependent solely on the rate of degradation. Thus enzymes with rapid turnover rates reach new steady state levels faster than enzymes with slow turnover rates. Six hours after poly rI·rC treatment, the tryptophan 2,3-dioxygenase level was maximally depressed to 60% of the control value and returned to the control value within 24 hr. Catalase and cytochrome P-450 levels were maximally depressed to about 50% of the control value between 24 and 40 hr. They were still below control values after 96 hours. The effect of poly rI·rC on cytochrome b_5 levels was less pronounced than for the other hemoproteins. These differences in time courses of loss and recovery of the hemoproteins can be related to the half-lives of the enzymes. Of the hemoproteins studied, tryptophan 2,3-dioxygenase has the shortest half-life, 2–3 hr; it was depressed most rapidly by poly rI·rC and returned to its normal level most rapidly. Catalase, which has a half life of about 30 hr, and cytochrome P-450 took a much longer time to decrease and recover.

The depressant effect of tilorone on liver hemoproteins was similar to that of poly rI·rC except that its onset was delayed by about 10 hr. This may relate to the delayed induction of interferon by this agent. Tilorone induces interferon 12–24 hr after its administration to mice (24) whereas poly rI·rC causes one peak induction of interferon at 2 hr and another at 9–12 hr after its administration (25–27). This delayed response to tilorone is probably not due to the time required for its absorption from the gastrointestinal tract because interferon induction was also delayed after intraperitoneal injection of the drug.

Both poly rI·rC and tilorone caused a depression of ALA-S activity, but only poly rI·rC elicited the early increase in holo tryptophan 2,3-dioxygenase formation. This would seem to contradict the view that the increase in holoenzyme is a reflection of an increase in the heme pool or that an increase in the size of the heme pool depresses ALA-S. However, it is possible that the increase in holoenzyme reflects only the increase in the size of the heme pool above that required for the depression of ALA-S. Maximum heme oxygenase induction by poly rI·rC was reached within 2 or 3 hr of its onset, but 20 to 30 hr were required for maximum induction when tilorone was administered (Fig. 5). Assuming that the rate of induction of heme oxygenase is a function of the availability of excess heme, it can be concluded that tilorone increases the size of the heme pool to a lesser degree than poly rI·rC.

Daily administration of poly rI·rC for 4 days did not depress mitochondrial cytochromes a, b or c significantly; cytochrome c_1 levels were lowered by 20%. These cytochromes have relatively long half lives (4 to 6 days); conceivably significant losses would have been observed if the experiment had been extended over a longer time period.

Poly rI·rC had no significant effect on the cytochrome P-450 or the benzo[a]pyrene hydroxylase activity of the adrenal and intestinal mucosa (Table 2). Kidney cortex benzo[a]pyrene hydroxylase activity was decreased by 40%, but the cytochrome P-450 content was not affected. This may reflect either a selective inactivation of a species of cytochrome P-450 with a relatively high specific activity for benzo[a]pyrene or a loss of NADPH-cytochrome P-450 reductase activity.

The failure of repeated doses of poly rI·rC to lower cytochrome P-450 levels in the intestinal mucosa, adrenal and kidney could mean that regulatory mechanisms for the maintenance of steady state levels of this cytochrome in extrahepatic tissues differ from that of the liver and that these systems are not vulnerable to the effects of interferon-inducing agents. It could also mean that the half-lives of the cytochrome P-450s of extrahepatic tissues may be much longer than that of hepatic cytochrome P-450. However, this cannot be the case for the cytochrome P-450 of intestinal mucosa, which is known to turn over quite rapidly (28, 29).

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