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# Reduction of Rat Liver Microsomal Ribonuclease by Cycloheximide

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SUMMARY

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An inhibitor of mRNA translation, cycloheximide, reduced RNA degradation by hepatic microsomal ribonuclease in a dose-related manner. This decrease occurred prior to a measurable reduction in microsomal protein or oxidative demethylation activity. Graphical analysis of the time plot of ribonuclease activity decay following cycloheximide administration indicated that the half-life of the microsomal ribonuclease was 54 min. The reduction of ribonuclease activity by this inhibitor was not attributable to its direct effects, nor was the presence of the adrenal glands necessary. The results indicate that the short onset of microsomal RNase reduction produced by cycloheximide may be due to a more rapid turnover of this enzyme in comparison with other microsomal proteins.

## INTRODUCTION

The experimental use of cycloheximide (Actidione) is based on its ability to inhibit protein synthesis (1–3). Cycloheximide interferes with mRNA readout (4, 5) and inhibits amino acid incorporation into protein (6–10).

Previous investigators have also suggested that cycloheximide treatment may alter nucleic acid metabolism, since cycloheximide increases hepatic DNA levels (11) and inhibits incorporation of labeled RNA precursors into ribosomal or nucleolar RNA during inhibition of protein synthesis (12, 13). Cycloheximide administration has also been reported to promote accumulation of hepatic microsomal RNA (14) and to reduce RNA breakdown by ribonuclease of human amnion cells in culture (15).

In the present study the influence of cycloheximide administration on hepatic microsomal RNA degradation by RNase was determined in order to examine the mechanisms through which cycloheximide alters microsomal nucleic acid metabolism. The results of this investigation suggest that cycloheximide produces a rapid decline of microsomal RNase activity. This decrease occurs prior to any measurable reduction in either hepatic microsomal oxidative *N*-demethylation activity or microsomal protein. A comparison of the half-life of the microsomal RNase with those of other microsomal proteins suggests that RNase may turn over more rapidly.

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## MATERIALS AND METHODS

Chemicals used were analytical reagent grade or the equivalent. Highly polymer-

ized yeast RNA was obtained from Calbiochem. Intact or adrenalectomized adult male (100–130-g) Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, Mass.).

Rats were maintained on commercial laboratory chow and water ad libitum in a room with controlled temperature and alternating 12-hr periods of light and darkness. Adrenalectomized animals were given 1% NaCl solution to drink and were used 1 week following adrenalectomy. At the time of death the animals were stunned and decapitated, and their livers were perfused *in situ* with ice-cold 0.25 M sucrose and homogenized with a motor-driven coaxial homogenizer at 1000 rpm for 1 min with 4 volumes of ice-cold 0.25 M sucrose. Homogenates were centrifuged at 9000 × $g_{av}$ for 20 min at 4° in an International Equipment Company model HR-1 refrigerated centrifuge. Supernatants obtained were recentrifuged at 78,000 × $g_{av}$ for 160 min at 4° in a Beckman model L-2 preparative ultracentrifuge. The pellet obtained was rinsed twice with 0.25 M sucrose and assayed for RNase activity by a previously described spectrophotometric method (16). Demethylation of *p*-chloro-*N*-methylaniline was determined by the method of Kupfer and Bruggeman (17) as modified by Fuller *et al.* (18). Phenolphthalein β-glucuronidase activity of microsomal suspensions or whole homogenates was determined by the method described by Bergmeyer (19). Protein content of microsomal fractions was estimated according to Lowry *et al.* (20).

## RESULTS

The influence of increasing cycloheximide doses on hepatic microsomal RNase is shown in Table 1. Within 24 hr ribonuclease activity was reduced to 51% or 27% of control values following 1 mg/kg or 2 mg/kg of cycloheximide, respectively. Increasing the cycloheximide dose to 3 mg/kg did not produce a significantly greater reduction in RNase activity.

The time course of cycloheximide effect on microsomal RNase is shown in Fig. 1. A progressive decrease in microsomal RNase activity was produced following the administration of a 2 mg/kg dose of cycloheximide. Graphical analysis of the data obtained indicated that a 50% reduction in microsomal RNase activity occurred within 54 min. RNase activity was reduced to 17% of control activity by 120 min following administration of cycloheximide.

The reduction of RNase activity by cycloheximide occurred at doses which have been shown to inhibit microsomal protein synthesis by 69–75% (11). Furthermore, earlier studies showed that cycloheximide

TABLE 1

*Effect of increasing cycloheximide dose on microsomal ribonuclease activity*

| Cycloheximide dose[a] | Ribonuclease[b] | Percentage of control |
|---|---|---|
| *mg/kg* | $E_{260}$/20 min/mg microsomal protein | |
| 0 | 0.251 ± 0.029 | 100 |
| 1 | 0.129 ± 0.010[c] | 51 |
| 2 | 0.068 ± 0.007[c] | 27 |
| 3 | 0.057 ± 0.009[c] | 23 |

[a] Doses of cycloheximide were administered intraperitoneally to groups of six rats each, 24 hr before the animals were killed.

[b] Mean ± standard error.

[c] Significantly different from control ($p \leq 0.05$).

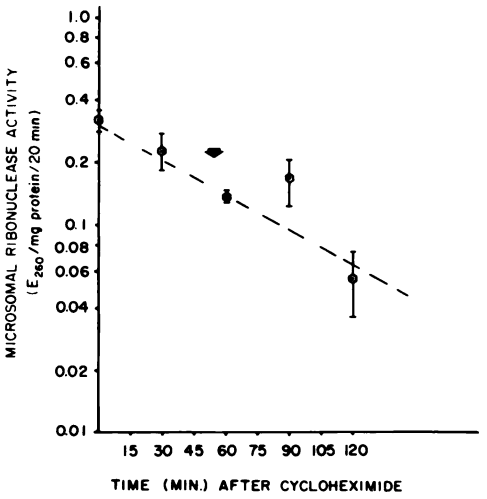


FIG. 1. *Time course of cycloheximide reduction of hepatic microsomal ribonuclease activity*

Values are the means ± standard errors of microsomal ribonuclease activity based on at least three rats per point. Male (160–200-g) rats were stunned and decapitated 30, 60, 90, or 120 min following administration of cycloheximide (2 mg/kg intraperitoneally). Distilled water was given to controls.

TABLE 2

*Reduction of liver microsomal ribonuclease following cycloheximide*

| Time[a] | Ribonuclease activity[b] | Percentage of control | *N*-Demethylase activity[b, c] | Microsomal protein[b] |
|---|---|---|---|---|
| *min* | $E_{260}$/20 min/mg microsomal protein | | *μmoles/30 min/g liver* | *mg/g liver* |
| 0 | 0.237 ± 0.024 | 100 | 5.94 ± 1.27 | 25.4 ± 2.0 |
| 60 | 0.074 ± 0.008 | 31 | 6.74 ± 1.20 | 24.1 ± 0.5 |
| 120 | 0.052 ± 0.004 | 22 | 7.33 ± 1.05 | 25.3 ± 1.3 |

[a] Liver 9000 × *g* or microsomal fractions prepared from at least three male (100–140 g) Sprague-Dawley rats per point after cycloheximide (2 mg/kg intraperitoneally) administration.

[b] Mean ± standard error.

[c] Formation of *p*-chloroaniline from *p*-chloro-*N*-methylaniline.

administration produced a 56% reduction in rat liver microsomal *N*-demethylase activity within 24 hr (21). These results suggest that the effect of cycloheximide on RNase may be due to a general inhibition of microsomal protein synthesis. Table 2 compares microsomal protein levels, *N*-demethylase activity, and RNase activity following cycloheximide administration. RNase activity was reduced to 31% and 22% of control values at 60 and 120 min, respectively, following cycloheximide. However, similar reductions in microsomal protein levels or oxidative *N*-demethylase activity were not observed up to 120 min following cycloheximide administration.

In order to determine the influence of administered cycloheximide on latent RNase activity, *p*-chloromercuribenzoate was added to pooled microsomes from control and cycloheximide-treated rats to inactivate endogenous microsomal RNase inhibitors (22, 23). The RNase activity of control microsomes was increased 1.3-fold by 1 mM PCMB[1] (Table 3). However, no RNase increase was produced when 1 mM PCMB was added to microsomes from cycloheximide-treated rats. Increasing the PCMB concentration to 2.5 mM produced 1.3- and 1.5-fold increases in RNase activity of microsomes from control and cycloheximide-treated animals, respectively.

The activity of a lysosomal "marker" enzyme, phenolphthalein β-glucuronidase (24), was compared in whole homogenates and microsomal preparations to assess the magnitude of lysosomal nuclease contribution to the RNase activity in these studies. Results in Table 4 suggested that 32% of the whole homogenate phenolphthalein β-glucuronidase activity was recoverable in the microsomal preparations. β-Glucuronidase activity of microsomes prepared following cycloheximide administration was measured to determine whether the RNase reduction was attributable to a decrease in lysosomal contamination following cycloheximide administration. The data presented in Table 4 suggest that lysosomal β-glucuronidase activity was not altered at 1 or 2 hr following cycloheximide administration.

The administration of cycloheximide at doses similar to those used in the present study was reported to increase adrenal weight and reduce adrenal ascorbic acid (14). These results suggested that cycloheximide-induced stress might mediate

[1] The abbreviation used is: PCMB, *p*-chloromercuribenzoate.

TABLE 3

*Ribonuclease stimulation by p-chloromercuribenzoate in liver microsomes of control and cycloheximide-treated rats*

Microsomes from rats given distilled water or cycloheximide (2 mg/kg intraperitoneally) and killed 1 hr later were pooled for duplicate determinations of ribonuclease activity.

| PCMB concentration | Ribonuclease activity | | Percentage of control |
|---|---|---|---|
| | Control | Cycloheximide | |
| *mM* | $E_{260}$/20 min/2 mg microsomal protein | | |
| 0 | 1.01 | 0.307 | 30 |
| 1.0 | 1.31 | 0.300 | 23 |
| 2.5 | 1.32 | 0.455 | 34 |

certain effects of this antibiotic, since adrenal steroid hormones have also been reported to reduce microsomal RNase activity (25). Cycloheximide (2 mg/kg intraperitoneally) was administered to adrenalectomized rats in order to determine whether the effect of cycloheximide on RNase required the presence of the adrenal glands. Microsomal RNase was reduced to 39% of controls 2 hr following the administration of cycloheximide to adrenalectomized rats (Table 5).

Sulfhydryl compounds have been reported to prevent the action of cycloheximide on peptide chain elongation in cell-free systems prepared from rat liver (5). These findings suggested that cycloheximide may exert direct effects on the activity of enzymes requiring intact sulfhydryl groups. In order to assess the possible contribution of direct effects of this inhibitor, cycloheximide (20 mg/ml) was incubated with pooled liver microsomes from untreated rats for 2 hr before the addition of RNA substrate. RNase activity of microsomes incubated with cycloheximide for 2 hr was reduced to 82% of control RNase activity. Thus direct cycloheximide effects did not account for the observed decrease in RNase activity.

TABLE 4

*β-Glucuronidase activity from liver whole homogenate and microsomal fractions following cycloheximide treatment*

| Source | Time after cycloheximide | β-Glucuronidase activity[a] | Percentage of whole homogenate |
|---|---|---|---|
| | *min* | | |
| Whole homogenate | 0 | 14.4 ± 1.7 | 100 |
| Microsomes | 0 | 4.6 ± 0.6 | 32 |
| | 60 | 4.5 ± 0.5 | 31 |
| | 120 | 4.3 ± 0.3 | 30 |

[a] Micromoles of phenolphthalein per 15 min per gram of liver equivalent obtained from at least three rats per point; mean ± standard error.

TABLE 5

*Reduction of liver microsomal ribonuclease activity by cycloheximide in adrenalectomized rats*

Rats were killed 2 hr after administration of cycloheximide (2 mg/kg intraperitoneally) or distilled water (to controls).

| Treatment | Ribonuclease activity[a] | Percentage of control |
|---|---|---|
| | $E_{260}$/20 min/mg microsomal protein | |
| Control | 0.114 ± 0.009 | 100 |
| Cycloheximide | 0.045 ± 0.006[b] | 39 |

[a] Mean ± standard error of six rats.

[b] Significantly different from control ($p \leq 0.05$).

## DISCUSSION

The results obtained in the present study indicate that cycloheximide treatment reduces RNA degradation by hepatic microsomal RNase. This reduction may be due to a decrease in microsomal RNase enzyme levels. These findings are in agreement with results of other workers (15), who reported a 25% decrease in RNase of human amnion cells in culture following cycloheximide addition. However, the magnitude of the reduction was less than that produced in the present study. Other investigators have reported that cycloheximide treatment enhances liver microsomal RNA accumulation (14). The present study suggests that reduction of RNA degradation may contribute to the accumulation of microsomal RNA (14).

The decrease in RNA degradation by microsomal preparations does not appear to be due primarily to direct effects of cycloheximide on the RNase enzyme, since the effect of this inhibitor *in vitro* was not comparable to that produced *in vivo*. Similarly, the decreased RNase activity could not be attributed to elevated levels of endogenous RNase inhibitor, since the addition of excess PCMB (to inactivate endogenous PCMB-sensitive RNase inhibitors) did not increase RNase activity to control levels.

Cycloheximide treatment was reported to modify the sedimentation characteristics of the hepatic endoplasmic reticulum (21). In the present study no changes in microsomal protein concentrations were noted during the 2-hr period following cycloheximide administration. Furthermore, the apparent contamination of the

microsomal pellets with lysosomal particles was not reduced by cycloheximide, since lysosomal β-glucuronidase activity was not appreciably reduced. These findings support the conclusion that the reduction of RNase levels was not attributable to a decrease in lysosomal RNase contamination.

Earlier investigators suggested that the enhancement by cycloheximide of amino acid incorporation *in vitro* requires the presence of the adrenal glands (26, 27). Other workers have reported that cycloheximide treatment increases adrenal weight and reduces adrenal ascorbic acid levels (14). These findings suggest that cycloheximide treatment may promote release of adrenal steroids. Since adrenal glucocorticoids have been shown to reduce hepatic microsomal RNA degradation (25), the possibility exists that cycloheximide might stimulate adrenal steroid release, which in turn would produce the observed reduction in RNase. The present findings indicate that the reduction of RNase by cycloheximide does not require the presence of the adrenal glands (Table 5).

Time course determinations of the effect of cycloheximide on RNase activity (Fig. 1) suggest that the half-life of this enzyme is 54 min. A comparison of the half-life of microsomal RNase with those reported by Schimke (28) for proteins of rat liver endoplasmic reticulum indicates that the half-life of microsomal RNase is less than that of other microsomal proteins. Thus the short onset of RNase reduction by cycloheximide may be due to a more rapid turnover of the microsomal alkaline RNase.

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