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NUTRITIONAL AND HORMONAL REGULATION OF LIVER DEOXYCYTIDINE KINASE ACTIVITY

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SUMMARY: Administration of CdR (50 mg/100 g every 12 h) elevated hepatic CdR kinase (EC 2.7.1.74) activity in normal or adrenalectomized rats over the control values 2- to 2.7-fold. Actinomycin (5 μg/100 g) or cycloheximide (50 μg/100 g) blocked the increase in enzyme activity. In starved rats, liver CdR kinase decreased to 64% compared to fed controls; refeeding restored enzyme activity to normal range. Steroid treatment (triamcinolone 1 mg/100 g/day) elevated liver CdR kinase activity to 138%, 169% and 193% of controls in 1, 2 and 3 days, respectively. Actinomycin or cycloheximide prevented this rise. These data suggest that the substrate and steroid induction of hepatic CdR kinase activity may be due to increased mRNA production and enhanced protein biosynthesis.

CdR kinase (deoxycytidine 5'-phosphotransferase; EC 2.7.1.74) phosphorylates deoxyribonucleosides and their analogs; it has broad substrate specificity and plays a role in the maintenance of normal deoxyribonucleotide pools. CdR kinase is an important salvage enzyme because it channels CdR into the heart of dNTP and DNA biosynthesis by yielding dCMP, a precursor of dCTP. The enzyme phosphorylates a variety of anti-neoplastic and anti-viral drugs (1-3). CdR kinase preferentially uses ATP as a phosphate donor (4). The activity was low in rat liver cytosol (0.8 nmol/h/mg protein); it increased 2- to 26-fold in 12 lines of chemically-induced, transplantable rat hepatomas of different growth rates (5). Kinetic properties of crude and purified preparations of CdR kinase have been examined (4-7); however, little is known about the nutritional and hormonal regulation of this liver enzyme. Here we report that hepatic CdR kinase activity is significantly elevated by CdR or steroid treatment. Actinomycin or cycloheximide prevents the substrate or hormonal induction of hepatic CdR kinase activity.

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Abbreviations: CdR, deoxycytidine; CdR kinase, deoxycytidine kinase; ACT, actinomycin; cyclo, cycloheximide; TAC, triamcinolone; ADX, adrenalectomized; dCMP, deoxycytidine monophosphate; dCTP, deoxycytidine triphosphate.

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MATERIALS AND METHODS

Animals. Adult male Wistar rats, weighing 130 to 200 g, were kept in separate cages and maintained on Purina laboratory chow and water ad libitum.

Chemicals and supplies. ATP, 2'-deoxycytidine, cycloheximide and actinomycin were purchased from Sigma (St. Louis, MO), 2'-deoxycytidine,[5^{3}H(N)]-(12-25 Ci/mmol) was from Moravek Biochemicals Inc., Brea, CA; polyethyleneimine cellulose plates (Polygram Cel 300 PEI) were from Brinkmann Instruments, Westbury, N.Y. TAC was from Indiana University Hospital Pharmacy. All other chemicals purchased were also of the highest available analytical grades.

Tissue preparation and biochemical studies. Rats were killed by stunning and decapitation. The preparation of homogenates and supernatant fluids and the determination of cellularity were reported (8).

CdR kinase activity. ^{3}H-CdR + ATP → dCMP + ADP was measured by the PEI cellulose plate method as described (6). Optimum enzyme kinetics were established and the assay was proportionate with enzyme amount added (up to 20 times) and time elapsed (up to 30 min). Optimum pH was 8.0. Activity was maximum at 0.4 - 0.6 mM CdR, with apparent K_m of 0.27 mM for hepatic cytosol preparation. Protein concentration was measured by a routine method with crystalline bovine serum albumin as standard. Enzyme activity was expressed in nmol per h per mg protein. In studies on starvation or TAC treatment where there is a marked shrinkage or enlargement of the liver, activity was expressed as nmol/h per average cell because the total liver cell number did not change under these conditions. Data are also given as percentages. Differences between means were subjected to statistical evaluation by the t test; those yielding a probability of less than 5% were considered statistically significant.

RESULTS AND DISCUSSION

Effect of CdR on hepatic CdR kinase activity. Time sequence study (Fig. 1). Rats were given a single dose of CdR (50 mg/100 g) i.p. and killed 0.5, 3, 6 and 12 h later. For the 24, 48 and 72 h groups, animals were given additional doses of CdR (50 mg/100 g) every 12 h. There was no change in hepatic enzyme activity up to 12 h; at 24, 48 and 72 h, activity was elevated 2-, 2- and 2.7-fold over the values of saline-injected controls. Doubling the dose of CdR (100 mg/100 g every 12 h) also provided similar increase in CdR kinase activity. Thus, hepatic CdR kinase is a substrate-inducible enzyme.

To test whether the adrenal glands play a role in induction of CdR kinase, ADX rats were treated with CdR (50 mg/100 g) every 12 h for 3 days. Hepatic CdR kinase activity was elevated to 294% by CdR in comparison to values of control ADX animals (from 0.32 ± 0.03 to 0.94 ± 0.06 nmol/h/mg protein) demonstrating that the substrate induction does not require the presence of the adrenals.

To throw light on the mechanism of the observed enzyme increases, groups of rats were treated for 72 h with CdR and also with actinomycin or cycloheximide. Since the substrate-induced increase of CdR kinase activity was prevented (Fig. 2), it is assumed that it is due to increased mRNA production and enhanced protein biosynthesis.

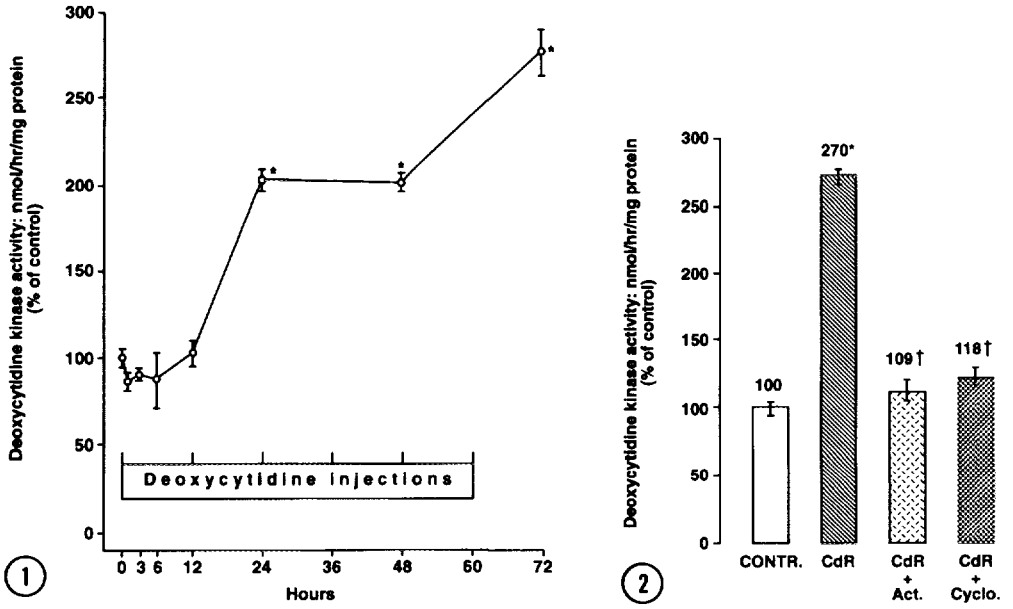

FIG. 1. Effect of CdR on hepatic CdR kinase activity. Each point shows means ± S.E. of 4 or more rats. Enzyme activity in liver of normal rat was 0.31 ± 0.02 nmol/h/mg protein. Results are expressed as % of controls.

*Significantly different from controls ($p < 0.05$).

FIG. 2. Actinomycin and cycloheximide prevented the CdR-induced increase in hepatic CdR kinase. Means ± S.E. are of 4 rats in each group. Enzyme activity in control was 0.33 ± 0.02 nmol/h/mg protein.

* Significantly different from controls ($p < 0.05$).

+ Significantly different from CdR-treated rats ($p < 0.05$).

Nutritional regulation of hepatic CdR kinase. Groups of rats (180-200 g) were starved for 3 days or starved for 3 days and refed for 2 days. As reported previously, in starvation while liver weight decreased, hepatic cellularity per g of tissue increased; both were restored upon refeeding. However, there was no significant change in total hepatic cellularity (8). In 3-day-starved rats CdR kinase activity in the average cell decreased to 64% compared to fed controls. When starved animals were refed for 2 days, enzymic values were restored to normal. Similar changes were noted in protein concentration in the average cell in starvation and refeeding (data not shown).

Effect of CdR on hepatic CdR kinase activity in starved and refed rats. Since CdR kinase responded to the nutritional status, we tested the effect of CdR in starved and refed rats. Fig. 3 shows that CdR administration to starved rats prevented the decrease in hepatic enzyme activity. When rats were starved for 3 days and then refed for 2 days and injected with CdR, the liver enzyme activity was elevated to 247% of control fed rats. When refed, CdR-treated animals were also given cycloheximide or actinomycin, the enzymic rise was blocked (data not shown).

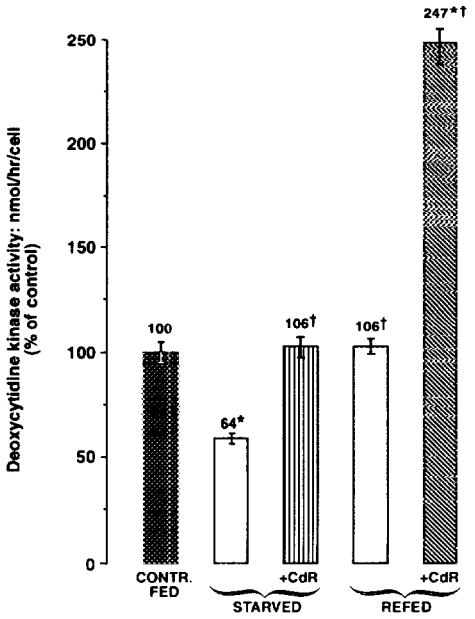

FIG. 3. Effect of CdR on hepatic CdR kinase activity in starved and refed rats. Means ± S.E. are for 4 rats in each group. CdR kinase activity in control was 17 ± 2 nmol/h/cell x 10^{-4} (0.44 ± 0.02 nmol/h/mg protein).
* Significantly different from controls ($p < 0.05$).
+ Significantly different from values of starved rats ($p < 0.05$).

Steroid regulation of hepatic CdR kinase. Since in starvation the plasma insulin level is markedly lowered but the steroid levels remain unaltered, the plasma steroid to insulin ratio is significantly elevated. To elucidate whether endocrine influences play a role in the control of liver CdR kinase, the effect of a synthetic corticosteroid, TAC, was studied. TAC (1 mg/100 g) was injected i.p. daily and rats were killed at 1, 2 and 3 days. During steroid treatment, since glycogen and lipids accumulate, liver weight increased to 130% of controls at 72 h. In contrast, hepatic cellularity per g of tissue decreased to 63% without significant alteration in total liver cellularity. These results agree with our previous data (9). At 1, 2 and 3 days of TAC treatment the activity of hepatic CdR kinase in the average cell was elevated to 138%, 169% and 193% of controls. The steroid induction of liver CdR kinase activity was prevented by actinomycin or cycloheximide given 30 min prior to TAC (data not shown). Together, these results suggest that both substrate and steroid hormone induction of liver CdR kinase activity may involve de novo enzyme biosynthesis.

The concept that hormonal and nutritional regulation of key rate-limiting enzymes in the synthetic and catabolic pathways is of pivotal importance in maintaining dynamic equilibrium is now widely accepted (10, 11). The expression of the genetic program after development is completed in an adult animal is influenced by nutritional and endocrine conditions for many hepatic enzymes such

as those involved in carbohydrate, lipid, ornithine and thymidine metabolism (12). In this study, we showed that the steroid- and CdR-induced increase in hepatic CdR kinase activity was prevented by actinomycin or cycloheximide. Since the activity of CdR kinase is elevated 5- to 30-fold in human carcinomas and cancer cell lines (6, 13, 14), further studies are in progress to elucidate various factors involved in the regulation of this salvage enzyme.

Novel aspects of this study. The results demonstrated that hepatic CdR kinase activity is induced by CdR in both normal and ADX rats. While starvation decreased enzymic activity, refeeding with or without CdR injections caused a significant enhancement. The fact that increases in liver CdR kinase activity seen with either the substrate or TAC treatment can be blocked by actinomycin or cycloheximide suggests that de novo enzyme biosynthesis is involved.

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