STIMULATORY EFFECT OF CYCLOHEXIMIDE AND RELATED GLUTARIMIDE ANTIBIOTICS ON LIVER URIDINE KINASE

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1. Introduction

Cycloheximide inhibits protein synthesis in a variety of mammalian cells, including hepatocytes, L-cells, and reticulocytes [1]. The drug prevents the transfer of amino acids from aminoacyl-tRNA to the growing polypeptide chain [2] and evidence has been obtained showing that cycloheximide inhibits both peptide initiation and extension by an effect on the donor site on ribosomes [3]. After the administration of cycloheximide the formation of ribosomes and the synthesis of nuclear 45 S RNA in rat liver are also decreased as a result of the reduced protein synthesis [4]. The mechanism of cycloheximide action is influenced by adrenal secretion [5]; in adrenalectomized rats no inhibition of amino acid incorporation into liver proteins has been observed [6].

Besides inhibiting protein synthesis cycloheximide increases tyrosine aminotransferase activity [7, 8]. It has been concluded that degradation as well as synthesis of the enzyme must be blocked in the cycloheximide-treated animals [9]. In the present report evidence is presented showing that another liver enzyme, uridine kinase, is influenced by the administration of cycloheximide and related glutarimide antibiotics.

2. Methods

The tested compounds were injected intraperitoneally to groups of 4–10 male rats (170–180 g), kept under standard conditions. At different time intervals thereafter the animals were killed by cervical dislocation and excised livers were homogenized in a glass homogenizer with a Teflon pestle with 3 vol of ice-cold 0.25 M Tris-HCl buffer, pH 7.5, containing 25 mM KCl and 5 mM Mg^{2+}-ions. Homogenates were centrifuged (10,000 rpm, 20 min, 2°) and the defatted supernatant fractions were used immediately for the estimation of enzyme activity.

Uridine kinase was assayed with 6-azauridine-2, 4-^{14}C (44 μCi/μmole) as a substrate [10] during a 10 min incubation period at 37° in 0.05 M Tris-HCl buffer, pH 7.5, with 12 mM adenosine 5′-triphosphate (Calbiochem) and 6 mM Mg^{2+} ions in a total volume of 0.3 ml in the presence of 0.1 ml of cell-free liver extract corresponding to 25 mg of liver wet weight. Analysis of the reaction mixture was effected chromatographically on a Whatman No. 1 paper in the solvent system composed of 1-butanol–acetic acid–water (10:1:3). The radioactive zones were cut out with respect to standards and their radioactivity was assayed with a Packard liquid scintillation spectrometer in 10 ml of scintillation fluid. The activity of uridine kinase was expressed as μmoles of phosphorylated 6-azauridine per 1 g of liver during 1 hr of incubation.

3. Results

During our studies on liver regeneration we found that the activity of uridine kinase can be increased several times by the administration of 5-azacytidine [11, 12]. As obvious from the data presented in table 1, a similar increase can be observed also after the administration of pactamycin, cycloheximide,

Table 1
Effect of various antibiotics on liver uridine kinase activity.

Administered compound	Dose level	Uridine kinase	
	(mg/kg)	(μmoles/gm/hr ± S.E.)	(%)
Control	0	0.98 ± 0.11	(100)
Actinomycin D (Merck)	1	0.88 ± 0.11	(90)
Mitomycin C (Kyowa)	5	0.92 ± 0.14	(94)
Vancomycin (Lilly)	50	1.03 ± 0.09	(105)
Streptomycin (Jenapharm)	50	0.97 ± 0.07	(99)
Fusidic acid (Løvens Kem. Fabrik)	2.5	1.01 ± 0.08	(103
Streptimidon (Dr. Z. Vaněk)	2.5	1.19 ± 0.14	(121)
Gougerotin (Calbiochem)	2.5	1.38 ± 0.19	(141)
Cycloheximide (Calbiochem)	2.5	2.00 ± 0.20	(204)
Streptovitacin A (Dr. Z. Vaněk)	0.2	2.17 ± 0.12	(222)
Pactamycin (Dr. W. Kersten)	2.5	2.30 ± 0.32	(235)

The compounds were administered i.p. to groups of 5–8 male rats (170–180 g) 24 hr before killing. The incubation mixture for the determination of uridine kinase activity in cell-free liver extracts is described in Methods.

and related glutarimide antibiotics. The action of pactamycin and especially of streptovitacin A (4-hydroxycycloheximide) is more pronounced than the effect of cycloheximide.

The time course of the enhancement of uridine kinase activity after the administration of cycloheximide and streptovitacin A indicates a 6–8 hr lag-phase preceding the actual increase of enzyme activity (fig. 1). Starting 28–30 hr after the administration of the drugs, there is no further increase of the enzyme activity, and approx. 48–54 hr after the drug administration the activity decreases to the level present in the control liver. The increase of uridine kinase in relation to the applied dose level of both compounds is shown in fig. 2. The administration of streptovitacin A brings about a rapid increase of uridine kinase ac-

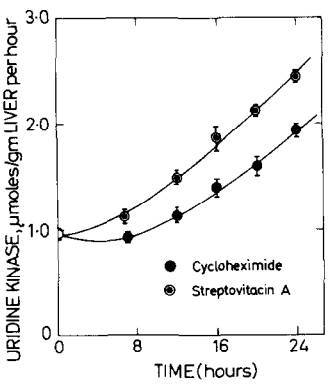

Fig. 1. Uridine kinase activity in the liver of rats treated with cycloheximide (1.5 mg per kg) and streptovitacin A (0.3 mg per kg). The compounds were administered i.p. to groups of 4–6 male rats (175–180 g); at different time intervals, thereafter the animals were killed and the activity of uridine kinase in cell-free liver extracts was assayed as described in Methods.

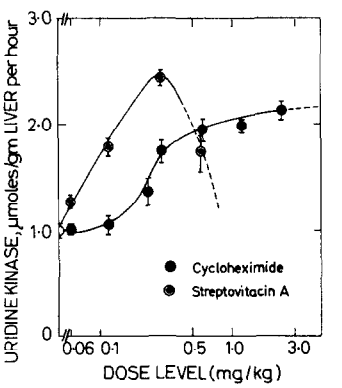

Fig. 2. The increase of liver uridine kinase activity in relation to dose level of administered glutarimide antibiotics. The compounds were administered i.p. to groups of 4–6 male rats (170–180 g) 24 hr before killing. The activity of uridine kinase was assayed as described in Methods.

Table 2
Stability of cycloheximide-mediated increase of liver uridine kinase.

Administered compounds	Dose level (mg/kg)	Uridine kinase (μmoles/gm/hr ± S.E.)	Increase (%)
Control	0	1.06 ± 0.13	100
Cycloheximide	2	1.94 ± 0.21	183
5-Azacytidine (5-AzCR)	10	1.76 ± 0.17	166
Cycloheximide + 5-AzCR	2 + 10	1.97 ± 0.16	185
Puromycin aminonucleoside	90	0.78 ± 0.12	74
Cycloheximide + Puromycin aminonucleoside	2 + 90	1.56 ± 0.22	147

The compounds were administered simultaneously via the intraperitoneal route to groups of 5–10 male rats (175 g) 24 hr before killing. The determination of uridine kinase activity is described in Methods.

tivity which reaches a maximum at the dose level of 0.3 mg of the drug per kg. Higher doses of the antibiotic are toxic [13]; this is obviously related to the irreversible inhibition of protein synthesis after the application of the drug [14]. The administration of increasing doses of cyloheximide results in the enhancement of uridine kinase with a maximum at the dose level 0.8–4.0 mg per kg.

The data given in table 2 show that the increase of uridine kinase represents a relatively stable process. 5-Azacytidine resulting in a similar increase of uridine kinase [12], does not cause any additional increase of the enzyme activity when administered in combination with cycloheximide. Simultaneous application of cycloheximide and puromycin aminonucleoside leads merely to a negligible decrease of the activity of the enzyme.

4. Discussion

We followed the activity of liver uridine kinase after the administration of antibiotics which interfere with different metabolic processes in the cell (table 1). Actinomycin D [15] and mitomycin C [16] were employed as inhibitors acting at the DNA level. As inhibitors of protein synthesis affecting the larger subunit of prokaryotic and eukaryotic ribosomes gougerotin—an inhibitor of peptidyl transferase [17]—and fusidic acid, inhibiting the translocation [18] were used. The data given in table 1 demonstrated that only glutarimide antibiotics and pactamycin cause an increase of liver uridine kinase. The drugs inhibit protein synthesis in eukaryotic systems only affecting the larger ribosomal subunit; pactamycin acts on the smaller one [17].

The increase of uridine kinase activity, observed after the administration of cycloheximide, reminds the finding of increased tyrosine aminotransferase activity [7–9], in spite of the fact that uridine kinase is not an inducible enzyme. The extensive application of cycloheximide in the studies on enzyme induction in various systems points to the differential sensitivity of different enzymes to cycloheximide (see e.g. [19]). It appears that, in accordance to the results of Kenney [9], cycloheximide interferes similarly to 5-azacytidine [20] not only with the synthesis of enzymes but also with their degradation. The results obtained in studies on uridine kinase enhancement after the administration of 5-azacytidine provide evidence of a relatively long half-life of template RNA of uridine kinase in the liver [21]. This finding together with the low inhibitory effect of puromycin aminonucleoside on uridine kinase activity (table 2) is not in contrast to the assumed inhibition of uridine kinase degradation following cycloheximide which would lead to an increase of its activity.

The possible effect of the adrenal system and of the increased glucagon secretion on the increased activity of uridine kinase in the liver of animals treated with glutarimide antibiotics is under investigation.

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