INHIBITION BY α-AMANITIN OF INDUCTION OF TYROSINE TRANSAMINASE IN RAT LIVER BY CORTISOL

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1. Introduction

Administration of glucocorticosteroids to rats induces the *de novo* synthesis of some liver enzymes such as tyrosine transaminase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) [1]. The molecular mechanism of the induction process has been a matter of controversy. Control at the level of transcription [2], translation of messenger RNA [3] or of its transport from the nucleus to the cytoplasm [4] has been proposed. Most of the evidence has been obtained by indirect means i.e. by the use of inhibitors of RNA synthesis such as actinomycin D and 8-azaguanine. Unfortunately, the mode of action of some of these inhibitors is not completely clear thus leading to ambiguous conclusions. α-Amanitin, a cyclic octapeptide isolated from the toadstool *Amanita phalloides* [5] is a specific inhibitor of mammalian RNA polymerase [6–9]. It interacts directly with the enzyme protein and does not affect the template. Recently, we demonstrated that *in vivo* administration of α-amanitin to rats leads to a significant inhibition of both ribosomal and DNA-like RNA synthesis and to an almost complete inhibition of transport of labelled RNA, with the exception of transfer RNA, from the nucleus to the cytoplasm [10]. We have therefore used α-amanitin as a tool to investigate whether the induction of the tyrosine transaminase by cortisol is dependent on synthesis of new RNA or whether it is independent of transcription.

2. Methods

Four groups of Wistar BRII 120 g male rats adrenalectomized 3 days before use were treated as follows. One group received amanitin (120 μg dissolved in 0.3 ml 0.05 M tris buffer pH 7.9) and one hour afterwards, 2 mg cortisol phosphate in 0.2 ml 0.14 M NaCl. A second group received amanitin alone, a third cortisol alone and a fourth was used as control. One, four and seven hours after the start of the experiment, tyrosine transaminase activity of the liver was assayed by a radiochemical method [11]. Concurrent with the measurement of the tyrosine transaminase, amino acid incorporation of the 40,000 *g* supernatants was assayed *in vitro* as described in [12]. The results are shown in fig. 1 and table 1.

3. Results and discussion

Administration of cortisol, leads to a rapid stimulation of tyrosine transaminase activity (fig. 1). If the animals are pretreated with amanitin, hormonal induction of the enzyme is suppressed. Amanitin alone leads to a progressive decline of tyrosine transaminase level, below that of the control, which is restored to control level by injection of cortisol. These changes in enzyme activity are independent of effects of amanitin on protein synthesis by the ribosomes, since no significant effects of amanitin on amino acid incorporation into proteins by the 40,000 *g* supernatant from rat liver can be observed (see table 1). Seven hours after amanitin injection, a small inhibition of amino acid incorporation is observed, which cannot

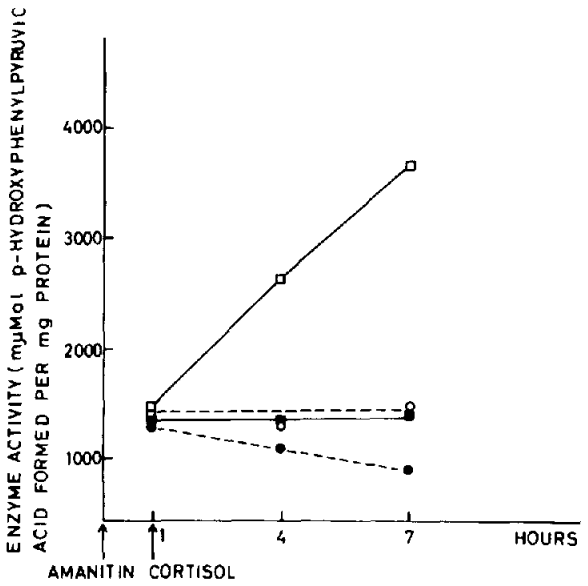

Fig. 1. Effects of α-amanitin on the induction of tyrosine transaminase in rat liver by cortisol. Rats were treated as described in the text with buffer, cortisol, amanitin or both and at the time intervals shown in the fig. sacrificed, the livers homogenized in 0.05 M tris-Cl buffer pH 7.55 containing 0.025 M KCl and 0.01 M $MgCl_2$, centrifuged at 800 *g* for 5 min, and then twice at 40,000 *g* for 20 min. The supernatants were used for the measurement of tyrosine transaminase activity using the radiochemical test described in [11].
○- - - -○ control
●- - - -● amanitin treated
□——□ cortisol treated
■——■ amanitin and cortisol treated

Table 1
Amino acid incorporation by rat liver 40,000 *g* supernatant from rats treated with amanitin and cortisol.

	Incorporation (cpm/mg protein)		
	1 hr	4 hr	7 hr
Control	185	190	212
Amanitin	192	165	188
Cortisol	–	257	242
Cortisol + amanitin	–	234	154

The supernatant obtained as described in fig. 1 was assayed for protein synthesis using the *in vitro* amino acid incorporation system described in [12] with ^{3}H-phenylalanine as the labelled amino acid.

quantitatively account for the observed suppression of enzyme induction. It should be mentioned that amanitin, in the dose used in our experiments, suppresses RNA synthesis up to 80–85% in the first 3–4 hr after application, whereas after 7 hr, inhibition is 20–30%. It is thus evident that α-amanitin, a specific inhibitor of RNA polymerase and of RNA synthesis, abolishes the hormonal induction of a liver enzyme. These results favour the hypothesis that the induction process, at least in the early phases, is dependent on the *de novo* synthesis of RNA, although complementary effects on other levels of control cannot be ruled out.

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