INHIBITORY EFFECT OF α-AMANITIN ON THE INDUCTION OF δ-AMINOLEVULINATE SYNTHETASE IN CHICK EMBRYO LIVER

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Received 16 April 1971

1. Introduction

Certain 5β-H steroid metabolites derived from natural hormones, and various exogenous chemicals produce experimental hepatic porphyria in the chick embryo which resembles in certain respects the hereditary human disease. In this experimental preparation porphyrin-heme synthesis is stimulated by these agents via the de novo induction of δ-aminolevulinate synthetase (ALAS), the rate limiting enzyme for the heme pathway [1–3]. The primary molecular mechanisms controlling this induction process are still under study; much of the evidence relating to these mechanisms has to date been obtained by indirect means utilizing various inhibitors of nucleic acid and protein synthesis [4, 5], the modes of action of which are complex or not yet completely understood.

In this study we have examined the effect of α-amanitin on the inducibility of ALAS in chick embryo liver to delineate a possible involvement of inducer agents specifically at the transcriptional level in the cell. α-Amanitin, the powerful toxin of the toadstool *Amanita phalloides* [6] is a specific inhibitor of mammalian RNA polymerase and has been shown to selectively inhibit one of the two RNA polymerases present in calf thymus and rat liver nuclei as well as one of the three present in sea urchin; the toxin does not react with bacterial RNA polymerases [7–9]. α-Amanitin is highly selective in its site of action; it does not react with the DNA template but interacts directly with the nucleoplasmic DNA-dependent RNA polymerase itself inhibiting synthesis of certain species of RNA by specifically blocking RNA chain elongation [10].

We present evidence here that α-amanitin inhibits the induction of ALAS in chick embryo liver by steroid and chemical inducers of this enzyme thus supporting the idea that transcription of certain species of RNA molecules is a necessary step in the induction process which these agents elicit in the cell [1, 2, 4].

2. Experimental

Four groups, in duplicate, of 17 day-old chick embryos were injected into the yolk sac as follows: one group received an inducer agent, either the 5β-H steroid etiocholanolone or the chemical, allylisopropylacetamide (AIA) solubilized in 0.2 ml of propylene glycol; the second group received a solution of α-amanitin 1 hr prior to administration of the inducers; the third and fourth groups were controls with solvent alone or α-amanitin with solvent. The eggs were then incubated at 37° for various periods of time following which the embryos were killed by decapitation, the livers removed, pooled according to groups and homogenized, and ALAS activity determined by measuring the amount of ALA produced in the reaction as previously described [11]. α-Amanitin was a generous gift of Professor T. Wieland.

3. Results and discussion

Control chick embryo liver showed a very low level of ALAS activity, as previously noted [3]; after administration of the inducers however, the

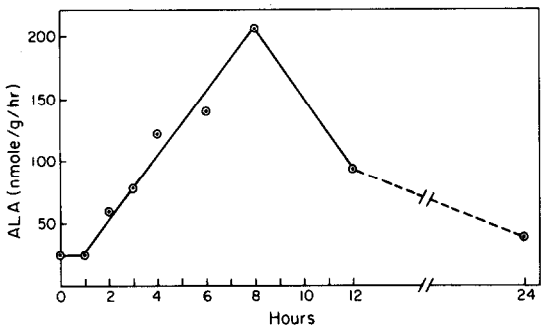

Fig. 1. Induction of ALAS activity in 17 day-old chick embryo liver after a single injection of etiocholanolone. Eggs were injected through the air cavity into the yolk sac with 3 mg of etiocholanolone dissolved in 0.2 ml of propylene glycol; controls receiving the solvent only showed no increase in enzyme activity over that shown at the 0-time. Each point represents the mean ALAS activity determined on pooled liver homogenates made up from 18 embryos.

enzyme activity increased substantially (fig. 1). The intensity of response to etiocholanolone was more variable than to AIA although detectable elevations of ALAS activity were noticeable within two hours after injection of the steroid. At 4 hr, ALAS activity had increased a minimum of 4–6 fold reaching a maximum at 8 hr, afterwhich the activity decreased rapidly, returning to control levels at 24 hr (fig. 1). If the embryos were pretreated with α-amanitin the steroid induction effect was markedly suppressed; in two different sets of experiments for example ALAS activity determined at 3 and at 4½ hr after administration of this inducer showed a 70% inhibition of enzyme induction (fig. 2a). The intensity of induction was more pronounced with AIA and the response usually lasted longer than 8 hr; these differences probably reflect slower metabolism and biological inactivation of this inducer as compared with the steroid. ALAS activity was increased to levels at least 10-fold greater than in control chicks after administration of AIA and in various inhibitor experiments α-amanitin diminished this response by 25% at 2½ hr, 43% at 3 hr and (fig. 2b) 55% at 4½ hr. Because α-amanitin appears to lose effectiveness after several hours [12] its inhibitory action on the induction response was not studied at longer time periods. The inhibitory effect of the toxin was dose-related with each inducer (fig. 3), but inhibition was never complete at the time periods studied.

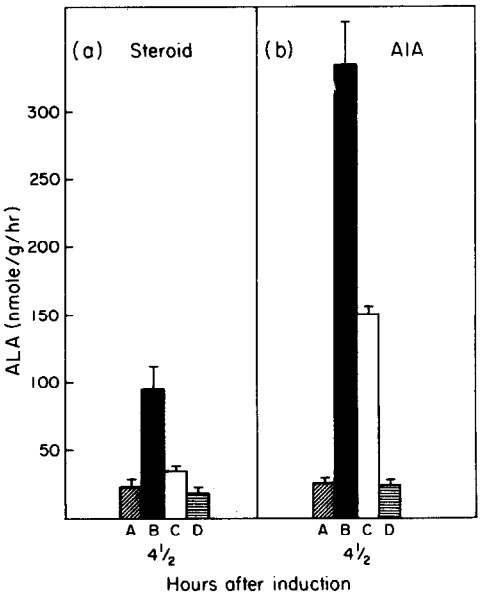

Fig. 2. Effects of α-amanitin on the induction of ALAS activity in 17 day-old chick embryo livers after administration of etiocholanolone (a) and AIA (b). Each column represents the average ALAS activity of a liver homogenate made up according to the various groups of embryos treated as described in the text: (A) controls injected only with propylene glycol, (B) induced with 2.5 mg of etiocholanolone or 3 mg of AIA dissolved in 0.2 ml of propylene glycol, (C) α-amanitin administered one hour prior to injection of the inducers, (D) α-amanitin and propylene glycol. At the time intervals indicated the embryos were killed by decapitation, 6 livers pooled per homogenate for each group in (a) and 4 in (b) and ALAS activity determined as described in the text.

The inhibition by α-amanitin of ALAS induction by both inducer agents was also studied in 16, 17 and 18 day-old chick embryo livers, since ALAS inducibility is known to increase with age when AIA* or etiocholanolone is used as the inducer in this experimental preparation. We found that the older embryos, while more readily induced by these agents, were also more sensitive to α-amanitin. The inhibitory effects of α-amanitin on ALAS induction in vivo were also reflected in parallel decreases in the incorporation of ^{3}H-uridine into RNA of induced chick embryo liver cells growing in primary tissue culture (unpublished observations). We have also

* A. Rifkind and P. Gillette, personal communications.

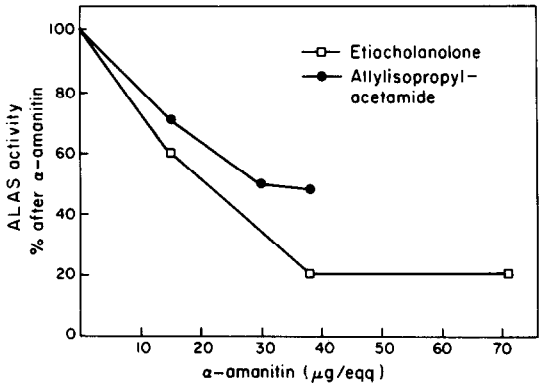

Fig. 3. Effect of varying concentrations of α-amanitin on the inducibility of ALAS activity by etiocholanolone and AIA in 17 day-old chick embryo livers. Eggs were injected as previously described under figs.1 and 2 with various concentrations of α-amanitin dissolved in 0.1 ml of 0.9% NaCl and administered one hour prior to administration of etiocholanolone (□——□) or AIA (●——●).

established that of the two RNA polymerases identifiable in chick embryo liver nuclei [13] it is the Mn^{2+}–$(NH_4)_2SO_4$ stimulated enzyme which is sensitive to this toxin, a finding which conforms to the results of other workers [7, 8, 14].

These findings indicate that the avian embryo is another experimental species in which enzyme induction elicited by steroids [3, 12] can be shown to be sensitive to the inhibitory action of α-amanitin. The inhibitory effect of α-amanitin on ALAS induction in the chick embryo thus implies the existence of some control mechanism operating at the transcriptional level in the liver cell which is responsive to the action of the wide variety of steroids, drugs and foreign chemicals which are known to induce the de novo formation of this enzyme.

Acknowledgements

The excellent technical assistance of Miss Maureen Bigley is gratefully acknowledged. This study was supported by U.S. Public Health Service Research Grant HD-04313.

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