FREE RIBONUCLEOSIDE TRIPHOSPHATES IN MOUSE LIVER AFTER α-AMANITIN INJECTION

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Received 9 January 1970

1. Introduction

α-Amanitin, a toxic polypeptide of *Amanita phalloides*, [1] impairs RNA synthesis in mouse liver [2] and when added *in vitro* to liver nuclei, it inhibits the RNA polymerase reaction activated by Mn^{2+} and high concentrations of ammonium sulphate [2]. According to Jacob, Sajdel and Munro [4], α-amanitin also inhibits [3] soluble RNA polymerase from rat liver. In order to ascertain whether α-amanitin affects RNA synthesis *in vivo* only by inhibiting ribonucleoside triphosphate polymerization or also by lowering precursor concentrations, the effects of this toxin on the amounts of ATP, GTP and UTP in mouse liver were studied in these experiments. Since the amount of CTP in mouse liver is very low and cannot be measured accurately, the effect of α-amanitin on the conversion of ^{3}H-cytidine into CTP was studied. The action of α-amanitin on the incorporation of ^{3}H-cytidine into RNA was also studied.

2. Methods

Male Swiss mice weighing 25–30 g were used. α-Amanitin was injected i.p. at a dose of 5 μg/10 g body wt. as a 0.005% solution in 0.9% NaCl. Control animals received an equal amount of saline and were killed at the same time as the poisoned animals, the experiments being run in parallel.

To determine the amounts of ribonucleoside triphosphates, mice were killed 1 hr after injection of α-amanitin. The livers from 4 mice were pooled, frozen in liquid nitrogen and lyophilized; the nucleotides were extracted, chromatographed and identified as described by Wegelin and Manzoli [5]. The DNA content of the pooled livers was determined according to Burton [6].

In the experiments on the conversion of cytidine into CTP, mice received an i.p. injection of ^{3}H-cytidine (50 μCi/ml in 0.9% NaCl; 5 μCi/10 g body wt.) 1 hr after α-amanitin administration. The mice were killed

Table 1
Incorporation of ^{3}H-cytidine into nuclear RNA.

Exp. no.	Time after ^{3}H-cytidine injection(min)	^{3}H-Cytidine μCi/10 g body weight	dpm of Nuclear RNA/mg DNA	
			Controls	Treated
1	30	5	178,537	66,384
2	30	5	159,447	80,931
3	60	3.5	280,227	80,386
4	60	5	483,028	206,419

Table 2
Free ribonucleoside triphosphates in the liver.

Expt. no	ATP (nmoles/mg DNA)		GTP (nmoles/mg DNA)		UTP (nmoles/mg DNA)		CTP (dpm/mg DNA)	
	Controls	Treated	Controls	Treated	Controls	Treated	Controls	Treated
1	272.7	400.0	47.5	58.7	34.3	36.8		
2	273.7	404.2	46.0	72.9	44.7	66.7	34,594	44,696

15 min later; the nucleotides were extracted and chromatographed and the radioactivity of CTP was measured.

In experiments on the incorporation into RNA, mice received an i.p. injection of ^{3}H-cytidine (35 or 50 μCi/ml in 0.9% NaCl; 3.5 or 5 μCi/10 g body wt.) 1 hr after α-amanitin administration. Thirty or sixty min after cytidine injection, the mice were killed, the livers from 4 mice were pooled and the nuclei isolated according to Widnell and Tata [7]. The nuclear RNA was extracted as described by Munro and Fleck [8] and its radioactivity measured.

3. Results

α-Amanitin strongly inhibits the incorporation of cytidine into RNA (table 1). It does not, however, inhibit the conversion of this nucleoside into CTP but slightly increases the amounts of ATP, GTP and UTP present (table 2). These results demonstrate that α-amanitin inhibits RNA synthesis in mouse liver by preventing only ribonucleoside triphosphate polymerization.

Acknowledgements

We are greatly indebted to Professor Th.Wieland, Heidelberg, for his generous gift of α-amanitin.

The experiments were supported by a grant from the Consiglio Nazionale delle Richerche, Rome.

References

[1] Th.Wieland, Science 159 (1968) 946.
[2] F.Stirpe and L.Fiume, Biochem. J. 105 (1967) 779.
[3] F.Novello, L.Fiume and F.Stirpe, Biochem. J., in press.
[4] S.T.Jacob, E.M.Sajdel, H.N.Munro, Biochem. Biophys. Res. Commun. 32 (1968) 831.
[5] I.Wegelin and F.A.Manzoli, J. Neurochem. 14 (1967) 1161.
[6] K Burton, Biochem. J. 62 (1956) 315.
[7] C.C.Widnell and J.R.Tata, Biochem. J. 92 (1964) 313.
[8] H.N.Munro and A.Fleck, Analyst 91 (1966) 78.