Effect of Allylisopropylacetamide on Nuclear Ribonucleic Acid Synthesis in Rat Liver

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The porphyrigenic drug allylisopropylacetamide, a potent inducer of δ -aminolaevulinatase synthetase, specifically increases nucleoplasmic RNA synthesis in rat liver. The drug-mediated increase in nucleoplasmic RNA synthesis is blocked by cycloheximide and haemin, which also inhibit the enzyme induction.

δ -Aminolaevulinatase, the first and rate-limiting enzyme of the haem-biosynthetic pathway, is known to be induced by a wide variety of foreign chemicals and endogenous steroid metabolites (Granick, 1966; Granick & Kappas, 1967). Induction of δ -aminolaevulinatase results in the overproduction of porphyrins and/or their precursors characterizing the different types of porphyrias (De Matteis, 1967). δ -Aminolaevulinatase induction by drugs can be prevented by the simultaneous administration of the inhibitors of protein and nucleic acid synthesis as well as haemin, the end product of the porphyrin-biosynthetic pathway (Granick, 1966; Marver *et al.*, 1966; Waxman *et al.*, 1966). Recent studies have implicated haem as a regulator acting at the level of translation (Sassa & Granick, 1970; De Matteis, 1971; Padmanaban *et al.*, 1973). However, no direct effect of allylisopropylacetamide or other porphyrigenic drugs on RNA synthesis in relation to enzyme induction could be detected (Granick, 1966; Sassa & Granick, 1970). In the present communication we report the preliminary findings which have provided evidence for the enhancement of nuclear RNA synthesis in response to allylisopropylacetamide.

Male rats (100–105 g) of the local Institute strain were starved for 48 h and then given a subcutaneous injection of allylisopropylacetamide (400 mg/kg). Haemin (20 mg/kg), cycloheximide (15 mg/kg) and actinomycin D (1.5 mg/kg), when

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given, were administered at the same time as allylisopropylacetamide by the intraperitoneal route.

Nucleolar and nucleoplasmic RNA synthesis were followed by measuring the incorporation of $^{32}\text{P}_i$ (carrier-free) or ^3H uridine (specific radioactivity 6200 mCi/mmol) obtained from Bhaba Atomic Research Centre, Trombay, India. They were given intraperitoneally at doses of 1.0 mCi and 300 μCi per 100 g body weight respectively. For the study of nucleolar and nucleoplasmic RNA synthesis the labelled precursors were administered 30 min after the drug administration. The animals were killed after 20 min and purified nuclei were isolated from liver homogenates pooled from three animals by the method of Blobel & Potter (1966). The procedure for the isolation of nucleolar and nucleoplasmic fractions described by Muramatsu & Fujisawa (1968) and the method of RNA extraction described by Abe *et al.* (1972) were followed. The nucleotide pool was isolated from liver homogenates by using HClO_4 to precipitate proteins and nucleic acids followed by adsorption of the nucleotides with charcoal. Radioactive samples were counted, in a Beckman LS-100 liquid-scintillation counter, in 10 ml of toluene containing 0.5% 2,5-diphenyloxazole. The experiments were repeated at least four times and the values given in Tables 1 and 2 represent those obtained in a typical experiment.

In the present investigation the effect of allylisopropylacetamide on nucleolar and nucleoplasmic RNA synthesis was investigated within 1 h after

Table 1. Effect of allylisopropylacetamide on nucleoplasmic and nucleolar RNA synthesis in rat liver

The experimental details are given in the text.

Treatment	Isotope used	Nucleotide pool (c.p.m./ E_{260} unit) (Y)	Nucleoplasmic RNA (c.p.m./ E_{260} unit) (A)	Nucleolar RNA (c.p.m./ E_{260} unit) (B)	10 $\times$ A/Y	10 $\times$ B/Y
Control	$^{32}\text{P}_i$	10150	5481	5075	5.4	5.0
Allylisopropylacetamide	$^{32}\text{P}_i$	9328	16603	3857	17.8	3.8
Control	^3H Uridine	500	265	165	5.3	3.3
Allylisopropylacetamide	^3H Uridine	456	907	105	19.9	2.3

Table 2. *Effect of haemin, actinomycin D and cycloheximide on allylisopropylacetamide-mediated increase in nucleoplasmic RNA synthesis*

Y is the specific radioactivity of the nucleotide pool. The experimental details are given in the text. [³H]Uridine was used as the labelled RNA precursor.

Treatment	Nucleoplasmic RNA (c.p.m./E ₂₆₀ unit) (A)	10 × A/Y
Control	265	5.3
Allylisopropylacetamide	907	19.9
Cycloheximide	274	5.6
Actinomycin D	102	1.7
Haemin	396	7.2
Allylisopropylacetamide + cycloheximide	370	7.4
Allylisopropylacetamide + actinomycin D	216	3.6
Allylisopropylacetamide + haemin	215	3.6

administration of the drug. The results presented in Table 1 indicate that allylisopropylacetamide causes a striking increase in nucleoplasmic RNA synthesis measured in terms of ³²P_i as well as [³H]uridine incorporation. The nucleolar RNA synthesis actually shows a decrease at this time-period. There is no change in the labelling pattern of the total nucleotide pool of the liver. To account for slight variations recorded in the labelling of the nucleotide pool with different treatments, the results have also been expressed as a ratio between the specific radioactivities of RNA and the nucleotide pool.

The results presented in Table 2 indicate that allylisopropylacetamide-mediated increase in nucleoplasmic RNA synthesis is blocked by actinomycin D, cycloheximide and haemin. Whereas actinomycin D blocks nucleoplasmic RNA synthesis in control animals as well, the cycloheximide and haemin effects are specific for the drug-mediated increase in nucleoplasmic RNA synthesis.

The results presented clearly establish that allylisopropylacetamide enhances nucleoplasmic RNA synthesis, which is the site for the synthesis of cytoplasmic mRNA and its precursors. That this effect may be specifically related to δ -amino-laevulinic synthetase induction is indicated by its susceptibility to the administration of haemin, which is the end-product 'co-repressor' for the enzyme. Further studies are needed to quantitatively relate the effect of allylisopropylacetamide on nucleoplasmic RNA synthesis and δ -aminolaevulinic synthetase induction.

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