INHIBITION OF VIRAL RNA SYNTHESIS BY LEVALLORPHAN

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Studies of the actions of narcotic alkaloids on bacterial and mammalian cells have shown that levallorphan and related compounds are potent inhibitors of RNA synthesis. At low concentrations, these drugs appear to inhibit RNA synthesis rather selectively [1, 2] and to induce in *Escherichia coli* a loss of cellular polyamines [3]. Higher concentrations lead to the inhibition of protein synthesis [4, 5] and produce a decrease in the intracellular levels of ATP [4]. The inhibition of RNA synthesis in *E. coli* has been reported to involve chiefly the various species of ribosomal RNA, including the 5 S component [6]; the formation of sRNA and the rapidly labeled, presumably mRNA are more resistant to the effect of the drugs. These observations all suggested the some aspect of nuclear functions or of genetic transcription, i.e. DNA-directed RNA synthesis, might be the primary site of action of levallorphan. Since the formation of RNA of some RNA viruses differs from that of the host with respect to the template, polymerizing enzyme and cellular localization, we have studied the effect of levallorphan on the RNA synthesis of an RNA virus, Mengovirus. The results show that the synthesis of Mengoviral RNA is at least as sensitive to levallorphan as host cell RNA synthesis.

Mouse fibroblasts (strain L-929) were maintained and propagated in Petri dishes using Eagle's medium, according standard procedures [7]. When the incorporation of radioactive precursors into nucleic acids was measured experiments were performed at a density of 2×10^5 cells per Petri dish (60 mm diameter).

The data in fig. 1 illustrate the effect of levallorphan on nucleic acid synthesis. RNA and DNA synthesis are nearly equally sensitive to the drug and are completely suppressed at 10^{-3} M levallorphan. These findings are similar to those previously reported by Greene and Magasanik at 5×10^{-3} M levallorphan [4]. These authors also reported that such a levallorphan concentration caused a depletion of the ATP level in HeLa cells. It therefore appeared important to establish whether the inhibition of DNA-dependent RNA syn-

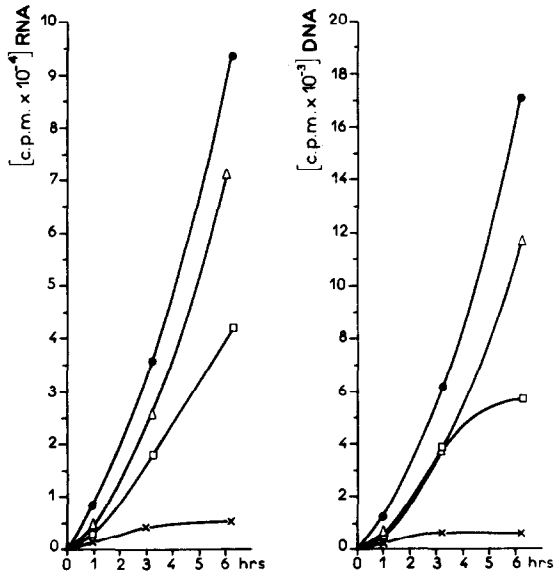

Fig. 1. Levallorphan is added to fibroblast monolayers (2×10^5 cells in 60 mm Petri dishes containing 5 ml of medium) followed after 15 min by ^{3}H-guanosine (1.7 μg/ml; 2 μCi/ml). At the indicated times the medium is removed, the cells washed and harvested. The incorporation of radioactive precursors into DNA (right) and RNA (left) is followed according to the procedure of Acs et al. [8]. Levallorphan: ●—● none; △—△ 2.5×10^{-4} M; □—□ 5×10^{-4} M; x—x 1×10^{-3} M.

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Table 1

Effect of levallorphan on free adenine nucleotide pool. Levallorphan 7.5×10^{-4} M was added to 8×10^5 plated cells (total incubation medium 3 ml) followed 10 min later by ^{3}H-adenosine 0.2 μg/ml, 2 μg/ml, 2 μCi/ml. After various periods of time, the cells still attached were washed five times with cold isotonic buffer to remove extracellular label and collected. The cells were washed again three times by suspension and centrifugation and suspended finally into 0.25 M perchloric acid for 30 min with occasional shaking. After centrifugation, 0.1 ml of the supernatant was mixed with 4 ml ethanol and 15 ml scintillation fluid and counted. Another aliquot of 0.1 ml was used for optical density measurement at 260 nm. 0.05 ml was used for paper electrophoresis, citrate-phosphate buffer pH 3.5, 2000 V, 90 min.

A: Total radioactivity (cpm/ml extract)

Nucleotide	Levallorphan	60 min	190 min	320 min
Total	0	21,930	23,640	16,153
	7.5×10^{-4} M	19,460	22,600	28,780

B: Distribution of radioactivity in adenine nucleotides

		cpm/0.05 ml extract	relative pool size AMP=1.00	cpm/0.05 ml extract	relative pool size AMP=1.00	cpm/0.05 ml extract	relative pool size AMP=1.00
AMP	0	742	1.00	968	1.00	615	1.00
ADP		1,257	1.69	1,114	1.15	928	1.51
ATP		2,220	2.95	1,618	1.67	1,074	1.74
AMP		336	1.00	897	1.00	617	1.00
ADP	7.5×10^{-4} M	849	2.52	1,350	1.51	1,160	1.88
ATP		1,408	4.18	1,654	1.73	1,850	3.00

Monolayers of L-cells (8×10^5 cells per Petri dish of 60 mm diameter) were incubated at 37°C in 3 ml of Eagle's medium containing 5% fetal calf serum. Levallorphan (7.5×10^{-4}M final concentration) was added; after 10 min incubation by ^{3}H-adenosine (final concentration 0.2 g/ml, 2 Ci/ml), Petri dishes were removed and processed at the indicated times. The incubation medium was aspirated, the monolayers washed three times with successive 3 ml aliquots of phosphate-buffered saline (PBS) (pH 7.2). The cells were detached from the surface of the Petri dish in the cold with a rubber policeman in the presence of PBS containing 0.02% trypsin, and harvested by centrifugation. The cell pellet was washed three times by recentrifugation from 5 ml aliquots of PBS at 0°C. The final pellet was resuspended in 0.3 ml of 0.25 M perchloric acid, maintained on ice with occasional shaking for 30 min, and recentrifuged. The UV absorption and radioactivity of the supernatant extract were determined; an aliquot (0.05 ml) was analyzed by high voltage electrophoresis, (0.05 M citrate-phosphate buffer, pH 3.5, 2000 V, 90 min) in the presence of known marker compounds. The zones corresponding to AMP, ADP and ATP were isolated and their radioactivity measured in a scintillation counter. The specific radioactivities of all adenine nucleotides were identical to within ± 50%; thus a comparison of the radioactivity in each fraction provides a measure of the relative sizes of the different pools. In the table these pools are all normalized to that of AMP which is arbitrarily taken as 1.00.

thesis could be dissociated from this effect. As seen in table 1, 7.5×10^{-4} M levvalorphan did not affect either the phosphorylation of adenosine, its conversion to AMP, ADP and ATP, or the relaive proportions of the various adenine nucleotides in the acid soluble pool.

To test for the susceptibility to levallorphan of virus directed functions, the cells were first pretreated with actinomycin and then infected with a high multiplicity of Mengovirus. Levallorphan was added either one or four hours after infection to permit the formation of at least some of the virus specific RNA synthetase in the latter case, and viral RNA synthesis was then followed by the incorporation of ^{3}H-uridine into RNA. As seen in fig. 2, concentrations of levallorphan which did not affect cellular ATP levels (5×10^{-4} to 7.5×10^{-4} M) strongly inhibited or suppressed the synthesis of viral RNA. At the few concentrations tested, the synthesis of viral RNA is inhibited by levallorphan at least to the same extent as that of RNA in uninfected cells.

We do not know the mechanism by which levallor-

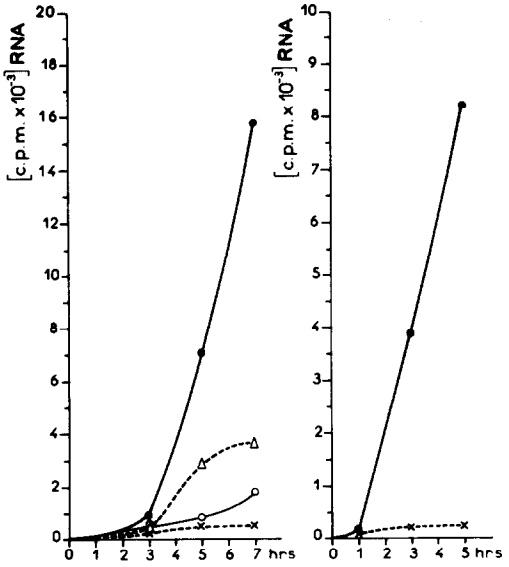

Fig. 2. Effect of levallorphan on Mengoviral RNA synthesis. Petri dishes (60 mm diameter) contain 2×10^5 fibroblasts in monolayer culture and 3 ml of culture medium. Actinomycin D (2–3 μg/ml) is added, followed after 20 min by a concentrated suspension of Mengovirus (10 pfu/cell). After permitting virus to adsorb for 1 hr, the medium is replaced by fresh medium containing actinomycin (2–3 μg/ml). Left: Directly after the change of medium levallorphan is added at the concentrations shown, followed immediately by ^{3}H-uridine (0.2 μg/ml, 2 μCi/ml) and thymidine (0.05 μg/ml). Right: The cultures are incubated for 3 hr after changing media, before addition of levallorphan, ^{3}H-uridine and thymidine as above. The cells are collected at 1,3 and 5 hr and the incorporation of radioactivity into material insoluble in 5% trichloracetic acid is measured in a scintillation counter. The time scale represents hours after the end of the virus adsorption period. *left* ●—● control Mego virus + actinomycin; ○—○ control actinomycin alone △—△ levallorphan 5×10^{-4} M; x—x levallorphan 1×10^{-3} M right ●—● control Mengo virus RNA + actinomycin; x—x levallorphan 7.5×10^{-4} M.

phan inhibits the formation of RNA by L-cells. However, the equal susceptibility of viral and cellular RNA synthesis suggests that the inhibition of nucleic acid synthesis is a secondary consequence of some other effect on the cell. A less likely alternative is that the synthesis of DNA, DNA-directed RNA and RNA-dependent RNA have certain intrinsic characteristics in common which render them equally susceptible to the action of the drug.

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