MOLECULAR PHARMACOLOGY, 9, 163–171

Action of Levallorphan: Macromolecular Synthesis and Cell Division

RALPH E. STEPHENS[1] AND ARTHUR M. ZIMMERMAN

Department of Zoology, University of Toronto, Toronto, Ontario, Canada

(Received July 31, 1972)

SUMMARY

STEPHENS, RALPH E., AND ZIMMERMAN, ARTHUR M.: Action of levallorphan: macromolecular synthesis and cell division. *Mol. Pharmacol.* 9, 163–171 (1973).

Tetrahymena pyriformis GL, an amicronucleate ciliate, was maintained axenically in a Proteose-Peptone medium and division was synchronized by the multiple heat shock method. Levallorphan, a non-narcotic analogue of morphine, was investigated over a range of concentrations from 0.01 to 5.0 mM for its effect on cell division and macromolecular synthesis. Increase in division delay and decrease in RNA, DNA, and protein synthesis were all determined to be directly related to the dose. Levallorphan reduced the amount and specific activities of polyribosomes labeled with [^{3}H]uridine and [^{14}C]amino acids. The synthesis of all species of RNA was reduced by levallorphan treatment, with ribosomal RNA being the most sensitive. These data support the proposal that the reduction in macromolecular synthesis following levallorphan treatment is directly related to the drug-induced division delays.

INTRODUCTION

Morphinans have been investigated in a variety of eukaryotic and prokaryotic systems (1). Most morphine analogues have been shown to inhibit growth whether turbidity, viable plating counts, cloning ability, or cell numbers are used as indices (2–6). The effects of these drugs, especially levorphanol and levallorphan, on macromolecular synthesis have also been extensively investigated (2, 7), but with apparently conflicting results. Simon and his co-workers reported that RNA synthesis was more sensitive to levorphanol and levallorphan than was either DNA or protein synthesis (5, 8, 9) and that ribosomal RNA synthesis was selectively inhibited over the synthesis of either transfer RNA or messenger RNA (5, 10). Utilizing different drug concentrations or different cell systems, others have reported that DNA and/or protein synthesis was equally subject to inhibition from treatment with morphinans (7, 11, 12).

Morphinans have been shown to affect active transport and other membrane-associated phenomena. Greene and Magasanik (13) have suggested that the action of these drugs on metabolic processes is due to energy losses caused by large, rapid effluxes of ATP and GTP from drug-treated cells. Other consequences of drug treatment have been changes in intercellular polyamine concen-

This work was supported in part by the National Research Council of Canada.

[1] Postdoctoral Fellow of the National Research Council of Canada. Present address, Department of Medicine, Division of Dermatology, Hahnemann Medical College and Hospital of Philadelphia, Philadelphia, Pennsylvania 19102.

trations (14, 15), phospholipid metabolism (16, 17), amino acid or nucleotide uptake (4), and nuclease activity (18). Most effects of morphinans on cellular structure and processes are readily reversible (2, 7).

Levallorphan is the non-narcotic, *N*-allyl-3-hydroxy analogue of morphine (19). Its relative strength with other morphinans in inhibiting growth and macromolecular synthesis has been established as follows: levallorphan > levorphanol > morphine (2, 3, 6).

Tetrahymena pyriformis serves as an excellent model system for studying the effects of drugs on cells. Effects on cell division are readily demonstrable in a system such as *Tetrahymena*, in which a high degree of division synchrony is readily induced, and analysis of drug effects on macromolecular events is facilitated by reference to the extensive background of biochemical and physiological data which have been accumulated on these cells (20, 21).

MATERIALS AND METHODS

Synchrony of organism. Tetrahymena pyriformis GL cells were maintained in nutrient medium (2% Proteose-Peptone with 0.1% liver extract) and division was synchronized according to Scherbaum and Zeuthen (22). The synchronization consisted of treating cells with a series of eight 30-min heat shocks (34°), each followed by a 30-min interval of growth at optimal temperature (28°). The end of the last heat shock (designated EH in certain figures) is the reference point for all experiments presented in this study. Results from division studies were obtained both from light microscopic counts

$$\left(\%\ \text{cells dividing} = \frac{\text{no. of cells showing division furrows on both sides}}{\text{total no. of cells}} \times 100\right)$$

and Coulter counts

$$\left(\text{Division index} = \frac{\text{final no. of cells} - \text{initial no. of cells}}{\text{initial no. of cells}}\right).$$

Cells were washed and transferred to inorganic medium (pH 6.8) 25–30 min prior to the last heat shock, at which time cell numbers could be adjusted for appropriate experiments. All experiments were carried out using inorganic medium prepared according to Hamburger and Zeuthen (23).

Preparation of drug. Levallorphan was graciously donated for these studies in the form of levallorphan tartrate (FW 433.4) by Hoffmann–La Roche, Montreal. Drug concentrations ranging from 0.01 to 5.0 mM were prepared in inorganic medium, and the pH of the final solution was adjusted to 6.8 ± 0.03. Slight variances in pH result in marked changes of the effectiveness of morphinans (3, 24); therefore careful attention was given to the standardization of pH. For all experiments levallorphan was added to the cells immediately after the last heat treatment, unless stated otherwise.

Total incorporation studies. Total incorporation experiments were carried out using 0.2 μCi/ml of [^{14}C]amino acid mixture, 2.5 μCi/ml of [^{3}H]uridine (specific activity, 10 Ci/mmole), or 2.5 μCi/ml of [^{3}H]thymidine (specific activity, 6.7 Ci/mmole), purchased from New England Nuclear Corporation. Radioisotopes were added 4–6 min after the last heat treatment. Duplicate 0.5-ml samples were taken at given intervals. The samples were immediately precipitated with equal volumes of cold 20% trichloracetic acid. The trichloracetic acid-insoluble material was then washed onto glass fiber filters and assayed for radioactivity.

Polyribosome studies. Between 35 and 50 min after the last heat shock levallorphan (2.5 mM) was added to the cells. Both [^{14}C]-amino acids (2 μCi/ml) and [^{3}H]uridine (10 μCi/ml) were added after 40–50 min. Drug- and/or radioisotope-treated cells were collected by centrifugation at 100 × *g* for 2 min and resuspended in 12 ml of inorganic medium (28°). The cells were again gently sedimented, rinsed once in 12 ml of 0.05 M Tris–5.0 mM $MgCl_2 \cdot 6H_2O$, buffer pH 7.4, at 28°, and resuspended in 4 ml of the same buffer at 4°. In order to lyse the cells this suspension was quickly added to 0.2 ml of sodium deoxycholate–bentonite solution (final concentrations, 0.3% and 0.25 mg/ml, respec-

tively) and continuously shaken for 10 min in an ice bath. The lysate was centrifuged at 12,000 × *g* for 10 min, and 3 ml of the resultant supernatant solution were layered onto 34 ml of a 15–30% linear sucrose gradient (sucrose solution prepared using the Tris-$MgCl_2$ buffer described above). The sample and gradient were centrifuged for 2.5 hr at 27,000 rpm in a Beckman model L3-50 ultracentrifuge with an SW 27.1 rotor at 4°. The gradient was collected in 1-ml lots with an ISCO fractionator. Absorbance at 254 nm was measured with a Zeiss PMQ II spectrophotometer, and radioactivity was determined.

Nucleic acid isolation and fractionation. Cells were simultaneously treated with drug (2.5 mM) and [^{3}H]uridine (20 μCi/ml) 35–50 min after the last heat shock. For comparison, control cells were treated only with the precursor [^{14}C]uridine (2 μCi/ml). The two groups of cells were mixed and chilled, and RNA was extracted with phenol for chromatographic analysis. In order to standardize the radioactivity between ^{3}H and ^{14}C, two additional cell cultures were treated with isotope 35–50 min after the last heat shock: one with [^{3}H]uridine and the other with [^{14}C]uridine. Using the ^{3}H:^{14}C ratio obtained from the standardization experiment, the counts of radioactivity from the [^{14}C]-uridine control and the [^{3}H]uridine drug-treated cells were adjusted to have equal heights (27).

Nucleic acids were extracted with phenol according to the method of Scherrer and Darnell (26), with modifications of Yuyama and Zimmerman (27) and McClean (25). Cells were washed once with 10 ml of cold sodium acetate, pH 5.1, containing 20 μg/ml of polyvinyl sulfate. Cells were then suspended in 2 ml of sodium acetate–polyvinyl sulfate solution to which was added sodium dodecyl sulfate (2% final concentration) in order to lyse the cells. After 10 sec an equal volume of cold sodium acetate–polyvinyl sulfate-saturated phenol was added to the lysate and shaken continuously for 5 min. The mixture was centrifuged for 10 min at 12,000 × *g*, and the resultant aqueous phase was removed. Two volumes of chloroform–isoamyl alcohol (24:1, v/v) were added to the aqueous phase, shaken for 1 min, and centrifuged for 2 min at 755 × *g*. The aqueous phase was again removed, and nucleic acids were precipitated by adjusting the final NaCl concentration to 0.1 M and treatment with 2 volumes of cold 95% ethanol. This mixture was then stored for 8–12 hr at −15° to ensure complete precipitation. The precipitate was then sedimented by 10 min of centrifugation at 12,000 × *g*, resuspended in 2 ml of suspension buffer (5 mM Tris, 1 mM EDTA, and 20 μg/ml of polyvinyl sulfate, pH 5.1), then reprecipitated by adding 2 volumes of cold 95% ethanol, and stored at −15°. For chromatographic analysis, the nucleic acid mixture was centrifuged for 10 min at 12,000 × *g* and the pellet was dissolved in 2 ml of 0.2 M NaCl–0.05 M sodium phosphate buffer, pH 6.8. The total sample was applied directly to the column in undiluted form and allowed to adsorb by gravity feed.

Methylated albumin Kieselguhr chromatography. Methylated albumin and methylated albumin-coated Kieselguhr were prepared according to the procedures of Mandell and Hershey (28) with a modification of Hayashi *et al.* (29). Methylated albumin Kieselguhr columns were prepared by packing 15 × 150 mm columns according to the methods of McClean and Warner (30) and McClean (25). Elution of the nucleic acids were carried out with 400 ml of a linear gradient of NaCl (0.2–1.2 M) at a flow rate of 1.7 ml/min at room temperature. The radioactivity and absorbance at 254 nm of each 2.5-ml fraction were determined.

Analysis of radioactivity. Radioactivity in each fraction was determined with a Packard Tri-Carb liquid scintillation spectrometer. Two methods of counting were used to accommodate either liquid samples or samples precipitated and dried on filter pads. Aliquots (0.5 ml) of fractions collected from methylated albumin Kieselguhr columns were added to 15 ml of scintillation fluid (5.5 g of Premablend I and 80 g of naphthalene dissolved in 410 ml of ethylene glycol monomethyl ether and 590 ml of toluene). For double labeling experiments the ^{14}C counting efficiency was 28% and the ^{3}H counting efficiency was 9% with a 35% spillover of

^{14}C activity into the ^{3}H channel. Filter pads containing material to be counted were placed in 15 ml of scintillation fluid (5.5 g of Permablend I and toluene to make 1 liter). Counting efficiency under these conditions for single labeling experiments was 72% for ^{14}C and 11% for ^{3}H.

RESULTS

Concentration-division studies. Levallorphan ranging in concentration from 0.01 to 5.0 mM was investigated for effects on division delay and division index. No effect upon either time, peak height, or division index of the first synchronous division resulted from treatment of the cells with 0.01 mM levallorphan. This concentration did, however, cause a 5-min delay in the second synchronous division. A concentration of 0.1 mM levallorphan produced an average first-division delay of 2.5 min and a second-division delay of 15 min. The division index also dropped below the control value of 0.98, to 0.80, for th first division. At 1.0 mM levallorphan the first division was delayed an average of 20 min but retained a relatively high division index of 0.70. Second division, however, was virtually eliminated. At a 5-fold greater concentration of levallorphan (5 mM) neither first nor second division peaks were observed, and the division index dropped to 0.06 (Figs. 1 and 2). If after 2–3 hr this division-blocking dose of levallorphan was removed by washing the cells in inorganic medium, the cells would have proceeded through division.

Delay in minutes was found to be directly related to the dose with the minimum threshold (no delay) between 0.05 and 0.10 mM and the maximum threshold (no division) between 1.0 and 5.0 mM levallorphan (Table 1).

Macromolecular synthesis. For these experiments, three concentrations of levallorphan were chosen which would effect (a) minimal division delay, (b) maximal division delay, or (c) complete inhibition of division. The effects of these concentrations of drug on the total incorporation of [^{14}C]amino acids into the trichloracetic acid-insoluble fractions were examined over a period of 2 hr after the last heat treatment (Fig. 3*A*). Control cells incorporated the amino acid

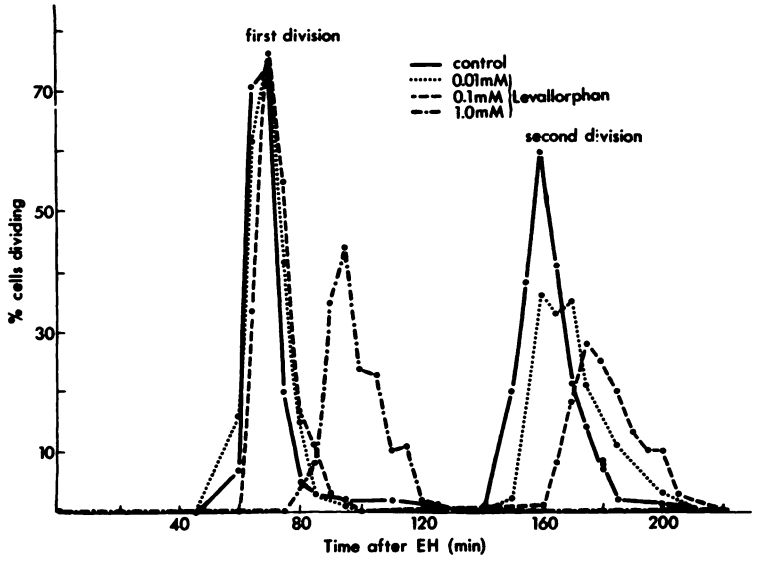

FIG. 1. *Effect of levallorphan concentration on first- and second-division profiles in division-synchronized Tetrahymena*

Division-synchronized *Tetrahymena* were continuously exposed to various concentrations of levallorphan, beginning immediately after the last heat treatment (0 min EH). Concentrations shown in this figure include 0.01, 0.1, 1.0, and 5.0 mM levallorphan. Aliquots of the cell suspension were removed at 5-min intervals, fixed, and observed microscopically for determination of percentage of cells dividing.

precursors at a fairly linear rate with typical deviations surrounding division times. At a concentration of 0.1 mM, which resulted in a 2.5-min delay of the first division, levallor-

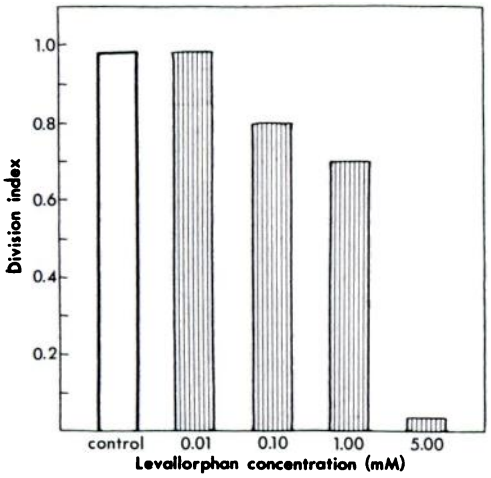

FIG. 2. *Effect of levallorphan concentration on division index of first division of cells following heat synchronization*

Division-synchronized *Tetrahymena* were continuously exposed to 0.01, 0.1, 1.0, or 5.0 mM levallorphan, beginning immediately after the last heat treatment. Duplicate 1.0-ml aliquots of the cell suspension were removed before (40 min after the last heat shock) and after (120 min after the last heat shock) the first division. Cell density was determined by use of a Coulter counter. The division index was calculated from the cell density as described under MATERIALS AND METHODS.

TABLE 1

Effects of concentrations of levallorphan on division time and division delay

Levallorphan concentration	Average division time	Average time delay
mM	*min*	*min*
Control	70.0 ± 2.7[a]	0 (5)[b]
0.01	70.0 ± 2.5	0 (4)
0.05	70.0	0 (2)
0.10	72.5 ± 3.5	2.5 (5)
0.25	75.0	5.0 (1)
0.50	77.5	7.5 (2)
0.75	85.0	15.0 (1)
1.00	95.0 ± 5.8	25.0 (4)
5.00	—[c]	(3)

[a] Standard deviation.
[b] Number of experiments.
[c] No division.

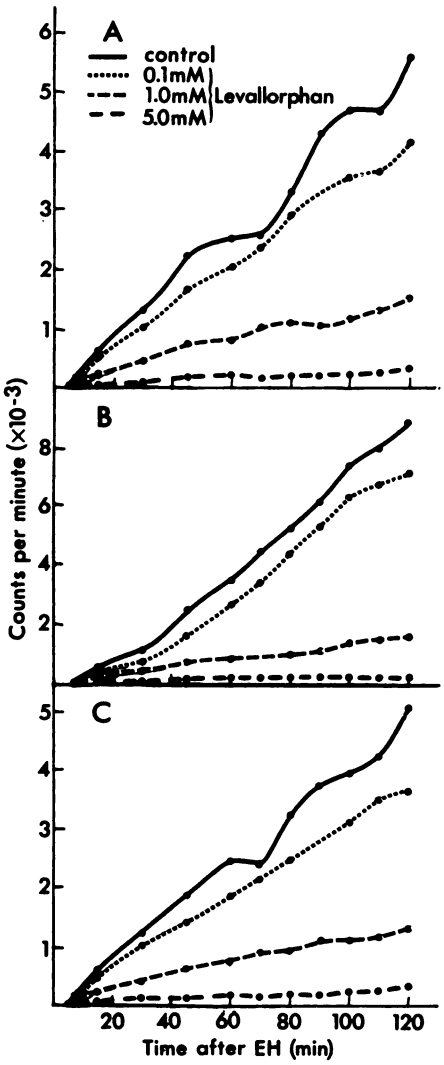

FIG. 3. *Effect of levallorphan concentration on macromolecular synthesis*

Cells were continuously exposed to 0.1, 1.0, or 5.0 mM levallorphan, beginning immediately after the last heat treatment (0 min EH). Appropriate isotopes were added 5 min later to the cultures for determination of their accumulative incorporation into protein, RNA, or DNA. Duplicate 0.5-ml aliquots were removed at 10-min intervals, precipitated with cold trichloracetic acid, and washed onto glass fiber filters for determination of radioactivity. The following precursors were used: for protein synthesis (A), [^{14}C]amino acids (2.5 μCi/ml); for DNA synthesis (B), [^{3}H]thymidine (10 μCi/ml); for RNA synthesis (C), [^{3}H]uridine (10 μCi/ml).

phan caused a slight but noticeable decrease in the incorporation of [^{14}C]amino acids. Higher concentrations produced even greater inhibition. The concentration which resulted in a maximum division delay typically reduced the total incorporation rate to about 25% of the control, while 5.0 mM levallorphan which completely blocked division, reduced the incorporation to the extent that total incorporation was barely discernible above background.

In the experiments designed to study the effects of drug concentration on DNA synthesis, [^{3}H]thymidine incorporation was considered to be an index of DNA synthesis. The resultant profiles of incorporation after treatment with 0.1 mM levallorphan were quite linear and paralleled the linear control curve, being only slightly depressed throughout the 2-hr interval. Treatment with 1.0 mM levallorphan depressed DNA synthesis to a slightly greater extent than protein synthesis. The total incorporation rate was approximately 15% of the control. Treatment with the higher concentration, 5.0 mM, allowed little or no incorporation of [^{3}H]thymidine (Fig. 3*B*).

The total incorporation of [^{3}H]uridine into control cells was linear with the exception of a lag during division. Treatment with 0.1 mM levallorphan produced only a slight decrease from the control, reminiscent of the results for protein synthesis. Increasing the concentration to 1 mM further reduced the incorporation to between 25% and 33% of the control. At 5 mM, levallorphan reduced the incorporation of [^{3}H]uridine drastically, almost to the point of complete inhibition (Fig. 3*C*).

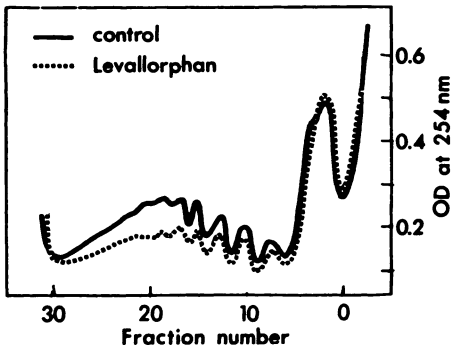

FIG. 4. *Comparison of polysomal material from equal numbers of control and levallorphan-treated cells*

Cells were subjected to a 15-min treatment with 2.5 mM levallorphan (35–50 min post-heat synchronization). Polysomes were isolated and layered in 3 ml on 34 ml of a 15–30% sucrose gradient. The sample was centrifuged for 2.5 hr at 27,000 rpm. Fractions of 1.0 ml were collected and analyzed spectrophotometrically for absorbance at 254 nm. Fraction 30 is the bottom of the gradient.

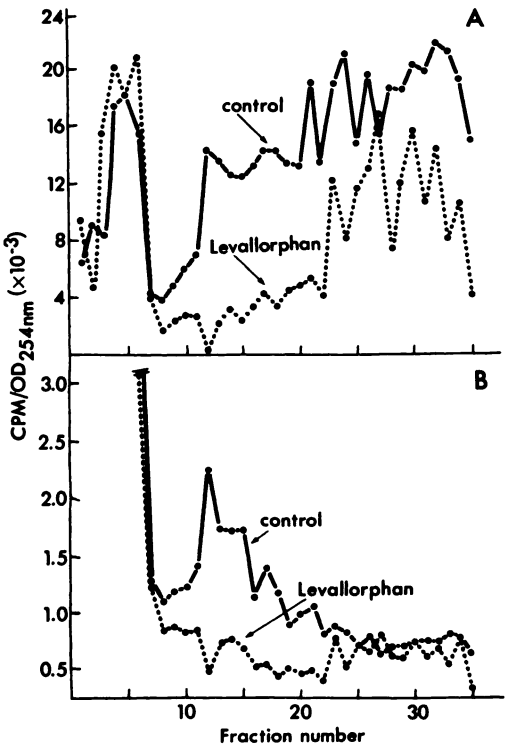

FIG. 5. *Effects of levallorphan on incorporation of radioisotopes into polysome-associated mRNA and nascent polypeptides*

Cells were treated with 2.5 mM levallorphan from 35 to 50 min after the last heat treatment and pulse-labeled for 10 min with radioisotopes (40–50 min after the last heat shock). Polysomes were isolated 50 min after heat shock and subjected to sucrose density analysis as described in Fig. 4. Fractions of 1 ml were collected, precipitated with cold trichloracetic acid, and washed onto glass fiber filters for determination of radioactivity. A. Specific activity of polysomes labeled with [^{3}H]-uridine (10 μCi/ml). B. Specific activity of polysomes labeled with [^{14}C]amino acids (2 μCi/ml). Specific activity = counts per minute/A_{254}. Fraction 35 is the bottom of the gradient.

Characterization of inhibition. Synchronized cells were treated with 1.0 mM levallorphan from 5 to 60 min after the last heat shock, after which polysomes were extracted and subjected to sucrose density gradient analysis. Polysomes were extracted from equal numbers of control and drug-treated cells. There was little difference between the amount of recoverable monosomes present in the treated and the control preparations, but the amount of polysomes recovered was greatly reduced in the treated fractions. Figure 4 shows a typical comparison of the treated cells with the control.

The association of messenger RNA with ribosomes and the synthesis of nascent proteins were further investigated. Cells were pulse-labeled for 35–50 min after the last heat shock with 2.5 mM levallorphan and further treated with both [^{3}H]uridine and [^{14}C]amino acids 5 min after addition of the drug (40–50 min after the last heat shock). Polysomes were recovered and subjected to sucrose density gradient analysis. A marked reduction in the specific activity (counts per minute/A_{254}) was found in both the uridine- and the amino acid-labeled macromolecules (Fig. 5). The reduced specific activity of messenger RNA ([^{3}H]uridine-labeled) was especially evidenced in the lighter polysome region, and to a significantly less degree in the heavier polysome region (Fig. 5*A*). The specific activity of the nascent polypeptides ([^{14}C]amino acid label) was also decreased by this same short pulse of levallorphan treatment (Fig. 5*B*). The reduction, as above, was more dramatic in the lighter polysome region; the heavier region displayed a protein-synthesizing ability equal to or only slightly reduced from the control.

Continued evaluation of the RNA species affected was done by analyzing phenol-extracted nucleic acids on methylated albumin Kieselguhr columns. The cells were treated with 2.5 mM levallorphan and precursor simultaneously (35–50 min after the last heat shock). The addition of drug drastically reduced the synthesis of all species of RNA. The species of RNA least sensitive to levallorphan treatment were 4 S and 5 S

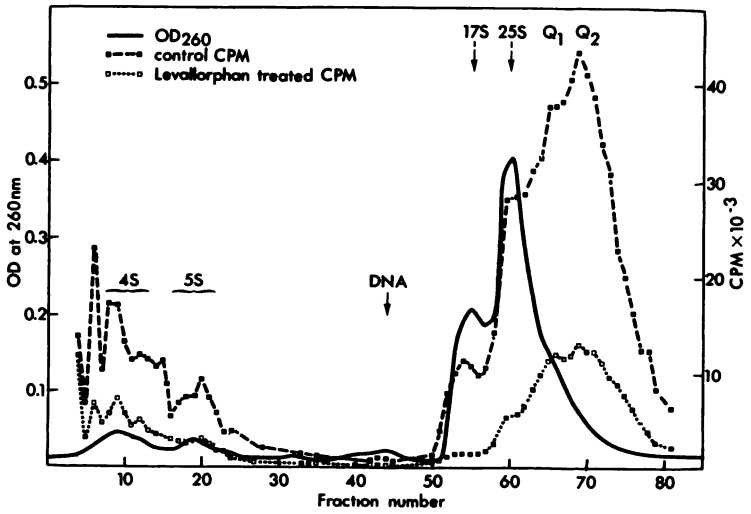

FIG. 6. *Characterization by methylated albumin Kieselguhr analysis of levallorphan effects on nucleic acid synthesis*

Cells were treated with 2.5 mM levallorphan and pulse-labeled with [^{3}H]uridine (20 μCi/ml) from 35 to 50 min after the last heat shock. Simultaneously, control cells were pulse-labeled with [^{14}C]uridine (2 μCi/ml). At 50 min after the last heat treatment control and treated samples were pooled and RNA was extracted by the cold phenol method. The sample was placed on a 15 × 150 mm methylated albumin Kieselguhr column and eluted with a linear gradient of 0.2–0.05 M NaCl in 0.05 M sodium phosphate (total, 400 ml at 2.5 ml/min). Fractions were analyzed for absorbance at 260 nm and radioactivity. ^{3}H and ^{14}C heights were adjusted from ratios obtained from an identical experiment excluding drug treatment.

RNA, which were reduced by more than 50%. Ribosomal precursor RNA (Q_1) and DNA-like RNA (Q_2) were reduced about 65–70%. The greatest inhibition was manifested by the 17 S and 25 S RNAs, which were reduced 80–85% (Fig. 6).

DISCUSSION

In division-synchronized *Tetrahymena*, levallorphan inhibits DNA, RNA, and protein synthesis. The synthesis of all RNA species is sensitive to levallorphan treatment, with rRNA synthesis being perhaps the most sensitive. Division delay in synchronized *Tetrahymena* is directly related to the concentration of levallorphan within the 0.05–5.0 mM range.

The results from our total incorporation experiments demonstrate the sensitivity of macromolecular synthesis to levallorphan treatment. The inhibition of RNA and protein synthesis is comparable within the range of concentrations reported herein (0.1–5.0 mM). These results with *Tetrahymena* agree with results obtained using levallorphan or levorphanol on *Escherichia coli* (5), *Bacillus subtilis* (15), *Staphylococcus aureus* (4), HeLa cells (11), chick embryo and rabbit kidney cells (31), and certain other mammalian tissues (7). Our results further show that DNA synthesis is at least as sensitive to levallorphan treatment as RNA synthesis or, as can be observed from experiments using 1 mM drug (Fig. 3), possibly more sensitive. Simon and his co-workers (5, 15) reported little if any DNA sensitivity of synthesis to levorphan or levallorphan in either *E. coli* or *B. subtilis*, but at higher drug concentrations Greene and Magasanik (13) found that in *E. coli* the synthesis of all three macromolecules was similarly sensitive. Insensitivity of DNA synthesis to levallorphan has also been demonstrated in HeLa cells (11); in the experiments by Roschenthaler *et al.* (8) DNA synthesis was determined to be so insensitive to levallorphan treatment that the DNA isotope ratio was used as an index for calculating the inhibition of other fractions. In contrast, Brdar and Fromageot (12) demonstrated that in mouse fibroblasts DNA and RNA are sensitive to levallorphan and both are completely suppressed by 1 mM levallorphan. The results of most investigations using a range of concentrations of either levallorphan or levorphanol on a variety of experimental material agree that RNA and protein syntheses are similarly sensitive, although some results indicate that RNA synthesis is slightly more sensitive. The contrast in results as to the sensitivity of DNA synthesis, from an equally wide range of materials, is as yet unresolved.

Experiments involving polysomal activity were carried out during the part of the cell cycle in which polysomes are readily assembled (32). A comparison of polysomal profiles from control and drug-treated cells showed a reduction in polysome content with little change of monosome content in cells which had been continuously treated with levallorphan at a concentration which significantly inhibits RNA synthesis (2.5 mM). These results are in good agreement with studies indicating polysome disaggregation in HeLa cells treated with levallorphan or levorphanol (6, 11, 33).

The entry of newly synthesized messenger RNA into the polysomal pool was dramatically reduced by a 15-min pulse of 5.0 mM levallorphan. These results, which are presented as the specific activity of polysomes, demonstrate a dramatic inhibition of the entry of new mRNA into the polysomal complex, especially in the lighter regions and to a lesser extent in the heavier regions. This concentration of levallorphan (5 mM), however, which practically eliminated RNA synthesis in the total incorporation experiments, did not entirely prevent the synthesis of messenger RNA or its subsequent entry into the polysomal complex. These results cannot delineate whether the specific activity was reduced because of reduced synthesis, transport inhibition, or inhibition of entry into polysomes.

There is also a decrease in the synthesis of nascent polypeptides on the polysomal complex. Even though incorporation of [^{14}C]-amino acids into nascent polypeptides was reduced, the translational process remained intact. Also, because protein synthesis is greatest in the same region which is most abundant with new messenger RNA (fractions 23–35), it is suggested that the new messenger RNA is capable of being utilized as a translational template.

Morphinans inhibit growth in many cell types (1). The present investigation, however, adds a new dimension to these studies, by definitely establishing that levallorphan can act upon division-synchronized *Tetrahymena* in a division-delaying or division-inhibiting manner without killing the cells. These results have further shown that between 0.01 and 5.0 mM levallorphan the delay is directly related to the dose. The relationship of the narcotic agents morphine and levorphanol to the division cycle has recently been established (25) and will be reported in a subsequent communication.

We have not meant to suggest that the manifestations of levallorphan on *Tetrahymena* presented in this paper are exclusively the direct result of drug treatment. The growing body of literature concerning membrane phenomena associated with drug treatment cannot be disregarded. Membrane-associated phenomena as well as stage-sensitive studies should be a fruitful line of research using division-synchronized and logarithmically growing cultures of *Tetrahymena*.

ACKNOWLEDGMENTS

The authors wish to express their sincere thanks to Dr. Daniel McClean and Mrs. H. Laurence for their technical assistance.

REFERENCES

1. D. H. Clouet (ed.), "Narcotic Drugs: Biochemical Pharmacology." Plenum Press, New York, 1971.
2. E. J. Simon, *in* "Narcotic Drugs: Biochemical Pharmacology" (D. H. Clouet, ed.), pp. 310–341. Plenum Press, New York, 1971.
3. E. J. Simon, *Science* **144**, 543–544 (1964).
4. E. F. Gale, *Mol. Pharmacol.* **6**, 128–133 (1970).
5. E. J. Simon and D. Van Praag, *Proc. Nat. Acad. Sci. U. S. A.* **51**, 877–883 (1964).
6. W. D. Noteboom and G. C. Mueller, *Mol. Pharmacol.* **5**, 38–48 (1969).
7. D. H. Clouet, *in* "Narcotic Drugs: Biochemical Pharmacology" (D. H. Clouet, ed.), pp. 216–228. Plenum Press, New York, 1971.
8. R. Roschenthaler, M. A. Devynck, P. Fromageot, and E. J. Simon, *Biochim. Biophys. Acta* **182**, 481–490 (1969).
9. E. J. Simon, *Nature* **198**, 794–795 (1963).
10. E. J. Simon and D. Van Praag, *Proc. Nat. Acad. Sci. U. S. A.* **51**, 1151–1158 (1964).
11. W. D. Noteboom and G. C. Mueller, *Mol. Pharmacol.* **2**, 534–542 (1966).
12. B. Brdar and P. Fromageot, *FEBS Lett.* **6**, 190–192 (1970).
13. R. Greene and B. Magasanik, *Mol. Pharmacol.* **3**, 453–472 (1967).
14. E. J. Simon, S. S. Cohen, and A. Raina, *Biochem. Biophys. Res. Commun.* **24**, 482–488 (1966).
15. E. J. Simon, L. Schapira, and N. Wurster, *Mol. Pharmacol.* **6**, 577–587 (1970).
16. E. F. Gale, *Mol. Pharmacol.* **6**, 134–145 (1970).
17. N. Wurster, P. Elsbach, J. Rand, and E. J. Simon, *Biochim. Biophys. Acta* **248**, 282–292 (1971).
18. R. K. Datta and W. Antopol, *Toxicol. Appl. Pharmacol.* **18**, 851–858 (1971).
19. A. F. Casy, *in* "Narcotic Drugs: Biochemical Pharmacology" (D. H. Clouet, ed.), pp. 1–16. Plenum Press, New York, 1971.
20. E. Zeuthen, *in* "Synchrony in Cell Division and Growth" (E. Zeuthen, ed.), pp. 99–158. Wiley, New York, 1964.
21. D. L. Hill (ed.), "The Biochemistry and Physiology of Tetrahymena." Academic Press, New York, 1972.
22. O. Scherbaum and E. Zeuthen, *Exp. Cell Res.* **6**, 221–227 (1954).
23. K. Hamburger and E. Zeuthen, *Exp. Cell Res.* **13**, 443–453 (1957).
24. E. J. Simon and P. Rosenberg, *J. Neurochem.* **17**, 881–887 (1970).
25. D. K. McClean, Cell division and macromolecular synthesis in *Tetrahymena pyriformis*: the action of tetrahydrocannabinol, morphine, levorphanol and levallorphan. Ph.D. thesis, University of Toronto, 1972.
26. K. Scherrer and J. E. Darnell, *Biochem. Biophys. Res. Commun.* **7**, 486–490 (1962).
27. S. Yuyama and A. M. Zimmerman, *Exp. Cell Res.* **71**, 193–203 (1972).
28. J. D. Mandell and A. D. Hershey, *Anal. Biochem.* **1**, 66–77 (1960).
29. M. Hayashi, M. M. Hayashi, and S. Spiegelman, *Biophys. J.* **5**, 231–246 (1965).
30. D. K. McClean and A. H. Warner, *Develop. Biol.* **24**, 88–105 (1971).
31. T. Rossman, F. F. Becker, and J. Vilcek, *Mol. Pharmacol.* **7**, 480–483 (1971).
32. J. Hermolin, Synthesis and localization of ribonucleic acid in synchronized *Tetrahymena pyriformis* GL. Ph.D. thesis, University of Toronto, 1971.
33. W. P. Summers, W. D. Noteboom, and G. C. Mueller, *Biochem. Biophys. Res. Commun.* **22**, 399–405 (1966).