PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 187-199 (1975)

The Inhibitory Effect of Theophylline on the Incorporation of ^{3}H-Deoxycytidine and ^{3}H-Thymidine into Thymocyte DNA (38502)

J. F. HOFERT AND R. H. LAUGEN
(Introduced by W. R. Ruegamer)

Department of Biochemistry, University of Nebraska College of Medicine, Omaha, Nebraska 68105

It is well known that methylxanthines display antimitotic properties *in vitro* (1). Theophylline has been shown to depress uptake of ^{3}H-thymidine into DNA of both fibroblasts (2) and phytohemagglutinin stimulated lymphocytes (3). Although uptake of labelled deoxynucleosides into acid precipitable material may reflect proliferative activity in cell cultures, we have shown that incorporation of ^{3}H-deoxycytidine into rat thymocyte DNA can be markedly suppressed by theophylline under conditions where there is no effect on the incorporation of ^{3}H-thymidine. Inhibition of uptake of ^{3}H-thymidine develops more slowly. These results indicate that incorporation of labeled deoxynucleosides into DNA of thymocytes should not be equated with DNA synthesis or cellular proliferation.

Materials and Methods. Sprague–Dawley derived male rats weighing 85–100 g were purchased from SASCO, Omaha, NB, and were maintained on Rockland Lab Chow and tap water *ad libitum* for at least 1 week prior to use. The animals were sacrificed by decapitation, and the thymuses were quickly removed, pooled, and placed in ice-cold minimal essential medium (MEM Joklik modified, Grand Island Co., NY) that had been gassed previously with 10% CO_2–90% air to bring the pH to 7.0–7.2. Cells were teased out of the thymus tissue from two to four rats (1.0–1.5 g), and the suspension was filtered through a 250 mesh stainless steel screen to remove the connective tissue. The thymocytes were washed three times at 0–4° with MEM using centrifugation at 600 *g* for 2 min. The cell suspension was made up to 3.0 ml, counted by standard hematological techniques, and diluted to 4.0×10^8 cells/ml.

Preincubations were performed in duplicate, under 10% CO_2–90% air at 37° and contained 0.2 ml of cellular suspension (8.0×10^7 cells), theophylline (Nutritional Biochemicals Corp. or Mann Research Labs) in 0.1 or 0.2 ml of MEM, and gassed MEM sufficient to bring the total to 2.0 ml (4.0×10^7 cells/ml).

Incorporations of ^{3}H-deoxycytidine or ^{3}H-thymidine (Schwarz Bioresearch, Inc., specific activities, 26.2 and 3.0 Ci/mmole) were conducted for 1 hr in triplicate using 0.5 ml of the preincubation mixture and 0.1 ml of the appropriate radioactive precursor containing 1.0 μCi dissolved in MEM. The incorporations were terminated by the rapid addition of ice-cold MEM containing 1×10^{-4} *M* nonradioactive deoxynucleoside. The cells were centrifuged, the supernatant aspirated, and the cell button washed with 5% TCA and collected on a nitrocellulose filter (Schleicher and Schuell, average pore size 0.45 μm). The filters were dried under a heat lamp and placed in 15 ml of scintillation fluid (4) for at least 18 hr prior to counting in a Nuclear-Chicago Unilux II liquid scintillation counter. Quenching was determined to be essentially constant for all tubes; therefore results are reported in cpm for the 2.0×10^7 cells counted. All glassware which came into contact with the cells during incubation or labelling was siliconized prior to use and washed in 7X nonionic detergent. (Linbro Chem. Co.)

Results and Discussion. Previous work had indicated that the incorporation of ^{3}H-deoxycytidine or ^{3}H-thymidine into thymocyte DNA in this system was linear up to 1 hour (5). Figure 1 shows that if control thymocytes were preincubated for 0–3 hr and pulsed for 1 hr with ^{3}H-deoxycytidine, uptake increased 21% after 1 hr of preincubation and then remained constant. Using cells that were not preincubated, theophylline (0.5 m*M*) depressed the incorporation of ^{3}H-deoxycytidine by 41%. The inhibitory effect did not change with incubation. In a separate experiment the rapidity of the theophylline effect was demonstrated by the fact that a theo-

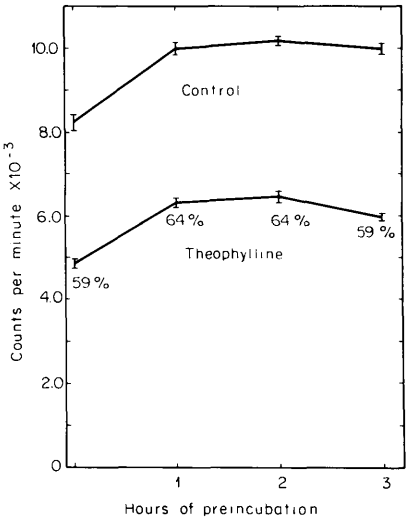

FIG. 1. Effect of varying preincubation time on the theophylline-induced depression of incorporation of ^{3}H-deoxycytidine into thymocyte DNA. Thymocytes were preincubated in 2.0 ml for the times shown without or with 0.5 mM theophylline, and then 0.5 ml of the medium (2.0×10^7 cells) was pulsed with 1.0 μCi of ^{3}H-deoxycytidine for 1 hr. Percentages reflect comparison of uptake with, to that obtained without theophylline. Vertical bars represent standard errors of the mean.

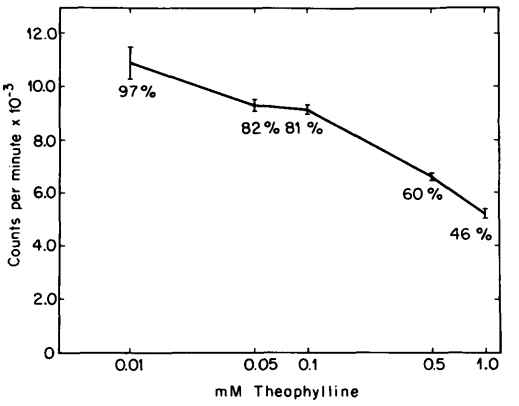

FIG. 2. Effect of varying the theophylline concentration on the incorporation of ^{3}H-deoxycytidine into thymocyte DNA. Cells were diluted to 2.0 ml in the presence of theophylline at the concentrations shown. The suspension (0.5 ml) was immediately pulsed, without preincubation, for 1 hr with ^{3}H-deoxycytidine. Percentages reflect comparison of uptake with, to that obtained without theophylline.

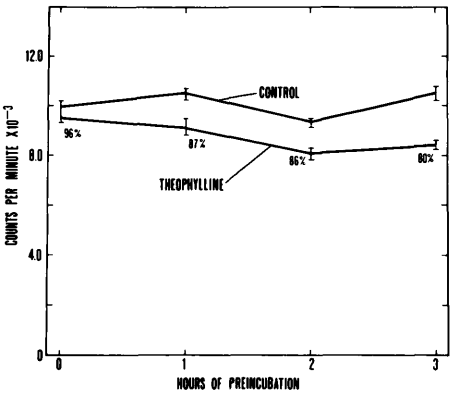

FIG. 3. Effect of 0.5 mM theophylline on the uptake of ^{3}H-thymidine into thymocytes. Experimental conditions were as in Fig. 1.

phylline concentration of 1.5 mM depressed the incorporation of ^{3}H-deoxycytidine into DNA 48% after 15 min of incubation.

Figure 2 shows the influence of varying the concentration of theophylline 100-fold on the uptake of ^{3}H-deoxycytidine. Theophylline at a level of 0.05 mM caused a significant depression of 18% ($P < 0.001$) while the highest concentration (1.0 mM) suppressed uptake 54%. Theophylline from two separate sources exhibited an identical inhibitory effect.

Although theophylline caused a rapid decline in incorporation of ^{3}H-deoxycytidine into the DNA of thymocytes, Fig. 3 demonstrates that 0.5 mM theophylline caused no depression of incorporation of ^{3}H-thymidine into the DNA of cells that were not preincubated. Uptake of ^{3}H-thymidine into the DNA of control cultures remained constant over the 3 hr preincubation period. The inhibitory influence of theophylline on ^{3}H-thymidine incorporation only became apparent after preincubation, and then increased slowly to a maximum of only 20% over the time period studied.

Because theophylline failed to inhibit the initial uptake of ^{3}H-thymidine, it may be concluded that the early suppression of ^{3}H-deoxycytidine incorporation cannot be due to an absolute depression of DNA synthesis but could be a result of any or all of the following factors: (a) suppression of the transport of ^{3}H-deoxycytidine into the cells; (b) an inhibition of a particular enzyme involved in the conversion of intracellular deoxycytidine into DNA such as a kinase or deoxycytidine

monophosphate deaminase; (c) an expansion of the endogenous pool of any metabolic intermediate between deoxycytidine and DNA.

Plagemann and Sheppard (6) have demonstrated that theophylline inhibits the transport of uridine and thymidine into rat hepatoma cells, but uptake of deoxycytidine was not tested. We have shown that 0.5 m*M* theophylline had no effect on the incorporation of ^{3}H-uridine into thymocyte nucleic acids.[1] The lack of an effect of 0.1 m*M* theophylline on uridine uptake into thymocyte nucleic acids had been reported previously (7).

It is of interest that a comparison of the effect of several inhibitory agents on ^{3}H-thymidine and ^{3}H-deoxycytidine incorporation into thymus DNA shows that uptake of ^{3}H-deoxycytidine is more readily depressed than is the uptake of ^{3}H-thymidine. Cortisol administration to rats *in vivo* caused a greater inhibition of incorporation of ^{3}H-deoxycytidine into thymus DNA compared with the uptake of ^{3}H-thymidine (8). Furthermore, thymocytes exposed to high oxygen tensions *in vitro* showed a rapid depression of incorporation of ^{3}H-deoxycytidine into the DNA but uptake of ^{3}H-thymidine was not suppressed (9). Perhaps there is a common explanation for these findings.

It is possible that theophylline exerts its metabolic effects on these cells directly or by increasing the intracellular cyclic-AMP concentration through its inhibitory effect on cyclic-AMP phosphodiesterase (10). Elucidation of the action of theophylline awaits further study.

Summary. In short term cultures of rat thymic lymphocytes, theophylline caused an early inhibition of incorporation of ^{3}H-deoxycytidine into DNA without influencing the incorporation of ^{3}H-thymidine. Over a 4-hr incubation period, the inhibitory effect of theophylline on ^{3}H-deoxycytidine uptake into DNA remained at approximately 40%, while the suppression of ^{3}H-thymidine uptake increased to 20%. The results indicate that since there is a difference in the effect of theophylline on the incorporation of ^{3}H-deoxycytidine and ^{3}H-thymidine into the DNA of thymus lymphocytes, inhibition of uptake of these deoxynucleosides by theophylline does not necessarily reflect inhibition of DNA synthesis or cell turnover.

This investigation was supported in part by PHS Grants No. CA 10291 and T12CA8021-03 from the National Cancer Institute. We are grateful to Mrs. Mary Alyce Vornholt for technical assistance.

[1] J. F. Hofert and R. H. Laugen, unpublished.

1. Deysson, G., Int. Rev. Cytol. **24,** 99 (1968).
2. Froehlich, J. E., and Rachmeler, M. J., Cell Biol. **55,** 19 (1962).
3. Hirschhorn, R., Grossman, J., and Weissman, G., Proc. Soc. Exp. Biol. Med. **133,** 1361 (1970).
4. Bray, G. A., Anal. Biochem. **1,** 279 (1960).
5. Hofert, J. F., Fed. Proc. **29,** 708 (1970).
6. Plagemann, P. G. W., and Sheppard, J. R., Biochem. Biophys. Res. Commun. **56,** 869 (1974).
7. Makman, M. H. and Cardenas, C. A., Fed. Proc. **29,** 616 (1970).
8. Hofert, J. F. and White, A., Endocrinology **82,** 767 (1968).
9. Hofert, J. F., Biochem. Pharmacol. **23,** 3216 (1974).
10. Butcher, R. W. and Sutherland, E. W., J. Biol. Chem. **237,** 1244 (1962).

Received July 18, 1974. P.S.E.B.M. 1975, Vol. 148.