Inhibition of RNA Synthesis in Salivary Glands of Drosophila melanogaster by 5'-Methylthioadenosine

Ronald E. Law, Ralph M. Sinibaldi, Michael R. Cummings and Adolph J. Ferro

Department of Biological Sciences
University of Illinois at Chicago
Chicago, Illinois 60680

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SUMMARY

5'-Methylthioadenosine (MTA) inhibits the incorporation of [^{3}H] uridine into RNA in salivary glands of Drosophila melanogaster. This effect is not due to an inhibition of [^{3}H] uridine uptake into the glands. The inhibition of RNA synthesis by MTA is concentration dependent and maximum inhibition is observed after 45 minutes of incubation in the presence of 1 mM MTA. Experiments utilizing α-amanitin suggest that the synthesis of heterogeneous RNA is completely inhibited.

INTRODUCTION

Concomitant increases in the levels of polyamines and in the accumulation or biosynthesis of RNA have been described during embryonic development (1-3); in regenerating rat liver (4,5); and during cellular response to hormonal stimulation (6-8). In Drosophila melanogaster, an increased concentration of spermidine was found to occur concurrently with increased RNA and protein synthesis during embryonic, larval, pupal and adult development (9, 10).

Synthesis of the polyamines spermidine and spermine is accompanied by the stoichiometric production of 5'-methylthioadenosine (MTA):

1) decarboxylated S-adenosylmethionine + putrescine → spermidine + MTA + H^+

2) decarboxylated S-adenosylmethionine + spermidine → spermine + MTA + H^+

Although numerous observations suggest that polyamines participate in the regulation of RNA synthesis, no information exists concerning the effect of MTA on RNA metabolism.

The polytene salivary gland chromosomes in D. melanogaster provide an excellent system for cytological, autoradiographic, and biochemical studies of RNA synthesis (11). Dion and Herbst (12) observed that spermidine is taken up by isolated Drosophila salivary glands and enters the cell nuclei. Addition of spermidine to the incubation medium resulted in a considerable enhancement in the incorporation of [^{3}H] uridine into the RNA of salivary gland nuclei. In this communication, we report on the effect of MTA on RNA synthesis in salivary glands of D. melanogaster.

MATERIALS AND METHODS

Stocks of Oregon R wild type Drosophila melanogaster were maintained at 25°C on an agar-cornmeal medium supplemented with yeast. 5'-Methylthioadenosine was prepared according to the method of Schlenk et al. (13). α-Amanitin was the product of Sigma. [5,6 ^{3}H] Uridine (40-60 Ci/mmole) was purchased from Schwarz-Mann.

Salivary glands of synchronized late third-instar larvae were dissected in 0.6% NaCl, transferred into 150 µl of [^{3}H] uridine (250 µCi/ml) in 0.6% NaCl and incubated with and without MTA in a final volume of 200 µl (pH 6.5) at 23°C for 1 hour. Incubations were terminated by transferring the glands to Whatman GF/C filters which were homogenized with 2 ml of cold 10% TCA in a Tenbroeck homogenizer. The homogenates were precipitated at 0-4°C for 30 minutes, and then collected on fresh Whatman GF/C filters. The filters were washed with 3 X 10 ml cold 10% TCA and 1 X 10 ml cold 95% ethanol. The filters were allowed to dry and the incorporation of [^{3}H] uridine into cold acid precipitable material was measured by counting in 5 ml of Triton X:toluene (1:2) in 0.5% PPO in a Beckman LS 250 scintillation counter. Uptake of [^{3}H] uridine into salivary glands was measured by immediately spotting 50 µl of the salivary gland homogenate on a Whatman GF/C filter which was dried and counted as previously described.

RESULTS

The uptake of [^{3}H] uridine into salivary glands explanted from late third-instar larvae is shown in Fig. 1. TCA soluble aliquots were assayed from glands incubated in the presence and absence of 1 mM MTA. The kinetics of [^{3}H] uridine uptake were similar in both control glands and those incubated in the presence of MTA.

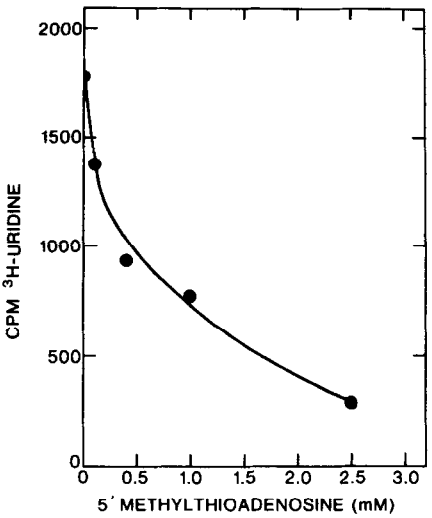

Fig. 1. Uptake of [^{3}H] uridine into explanted salivary glands from late third-instar larvae. Each point represents the uptake of label into five pairs of salivary glands as determined from a 50 μl aliquot of the TCA homogenate. Glands were incubated in 150 μl of [^{3}H] uridine (250 μCi/ml) at 23°C in a final volume of 200 μl. The uptake of [^{3}H] uridine into salivary glands was measured in the absence (●——●) or presence of 1 mM MTA (o——o). MTA was present in the incubation mixture at zero time.

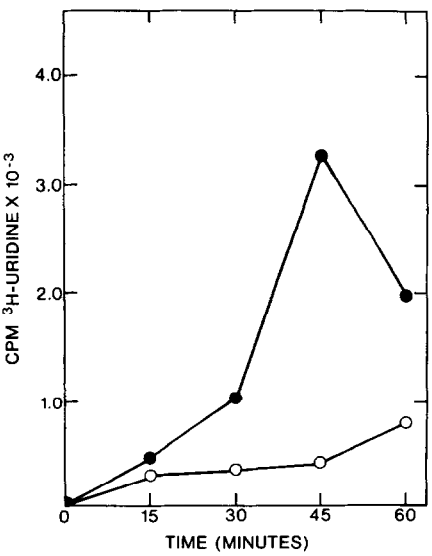

Fig. 2. Effect of MTA on the incorporation of [^{3}H] uridine into RNA in explanted salivary glands from late third-instar larvae. For each point, five pairs of salivary glands were incubated in the absence (●——●) or presence of 1 mM MTA (o——o). Incubation conditions were as described in the legend of Fig. 1.

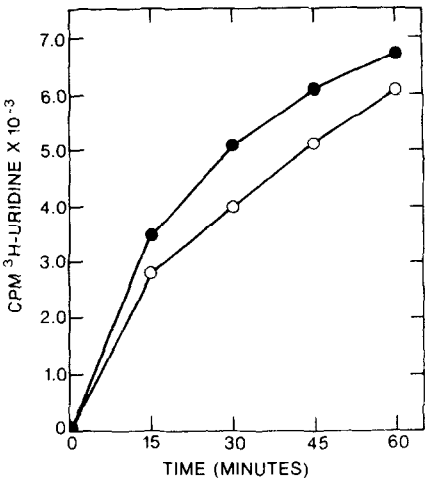

Fig. 3. Effect of increasing concentrations of MTA on RNA synthesis in explanted salivary glands from late third-instar larvae. For each point, three pairs of salivary glands were incubated in 150 µl of [^{3}H] uridine (250 µCi/ml) for 60 minutes at 23°C in a final volume of 200 µl.

The incorporation of [^{3}H] uridine into RNA of explanted salivary glands is shown in Fig. 2. The data are representative of replicate experiments except that some variation existed in the absolute levels of incorporation from one experiment to another. In control glands, incorporation increased during the first 45 minutes of incubation. At 60 minutes, however, a marked decrease was observed in the level of incorporation. Glands incubated in the presence of 1 mM MTA incorporated significantly less [^{3}H] uridine into RNA than the glands incubated in the absence of the nucleoside. The greatest degree of inhibition was observed at 45 minutes, when the presence of MTA resulted in an 88% reduction in the incorporation of [^{3}H] uridine into RNA.

Inhibition of [^{3}H] uridine incorporation into RNA as a function of the concentration of MTA is shown in Fig. 3. MTA was found to be inhibitory in the range of 0.1 mM to 2.5 mM. A maximum inhibition of 83% was observed in glands incubated for 60 minutes in the presence of 2.5 mM MTA.

TABLE 1

Effect of MTA and α-Amanitin on RNA Synthesis

Additions to Incubation Medium	cpm Incorporated	% Inhibition
None	2680	
0.1 μg α-amanitin	1400	47.8
1 mM MTA	737	72.5
0.1 μg α-amanitin + 1 mM MTA	750	72.1

For each experiment, 5 pairs of salivary glands were incubated for 60 minutes as described in Materials and Methods.

Incubation of explanted salivary glands in a low concentration of α-amanitin (0.1 μg/200 μl) provides information on the nature of the RNA synthesized during labeling. In eukaryotes, the synthesis of heterogeneous RNA is specifically inhibited by low concentrations of α-amanitin (14). Almost 50% of the RNA synthesized is α-amanitin sensitive, and is presumably heterogenous RNA (Table 1). The comparative inhibitory effects of α-amanitin and MTA on RNA synthesis are examined in Table 1. Glands incubated in the presence of 0.1 μg α-amanitin were inhibited 48% in the incorporation of [^{3}H] uridine into RNA, while those in 1 mM MTA were inhibited 73%. No additive effect in inhibition was observed when α-amanitin was added to an incubation medium containing 1 mM MTA.

DISCUSSION

We have observed that the naturally occurring compound, 5'-methylthioadenosine, is a potent inhibitor of RNA synthesis in D. melanogaster salivary glands. MTA also produces a minor inhibition in the total uptake of [^{3}H] uridine. This inhibition of uptake may be a consequence of the turn-off in RNA synthesis and by itself is of insufficient magnitude to account for the dramatic difference observed in the incorporation of [^{3}H] uridine into RNA in control and MTA-treated glands.

RNA metabolism in late third-instar D. melanogaster salivary glands has been studied in detail by Ellgaard and Clever (15). They also observed a decrease in [^{3}H] uridine incorporation into salivary gland RNA after 60 minutes incubation, and attributed this to the turnover of a rapidly degraded high molecular weight fraction. This agrees well with our results for [^{3}H] uridine incorporation in control glands. Additionally, they determined that only 20-30% of the RNA synthesized during a 60 minute incubation was pre-ribosomal. Our observation that incubation in the presence of α-amanitin and MTA was equivalent in inhibitory effect to that of MTA alone suggests that the synthesis of heterogenous RNA is completely inhibited by MTA.

It may be possible to reconcile the apparent antagonism of polyamines and MTA in their effect on RNA synthesis by considering the metabolic fate of MTA. In E. coli (16, 17) and certain animal tissues (18) MTA is cleaved by the 5'-methylthioadenosine nucleosidase to form adenine and 5'-methylthioribose. Ferro et al. (17) have suggested that in E. coli this enzyme is responsible for the observed low intracellular level of MTA.

Formation of MTA, independent of the polyamine biosynthetic pathway, can occur through the action of an S-adenosylmethionine cleaving enzyme (19-23). In this reaction, S-adenosylmethionine is cleaved directly into 5'-methylthioadenosine and homoserine. Physiological formation of MTA, therefore, is not exclusively coupled to the biosynthesis of polyamines.

Our observation that MTA inhibits RNA synthesis in salivary glands of D. melanogaster suggests that the intracellular metabolism of MTA may have important regulatory consequences.

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