Adenosine Selectively Inhibits Labeling of Chromosomal RNA, Especially hnRNA, Probably by Acting at or near the Site of Chain Initiation

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ABSTRACT The effects of adenosine on labeling of nucleolar preribosomal RNA, chromosomal plus nuclear sap hnRNA, and 4–5S RNA in explanted salivary gland cells of *Chironomus tentans* has been studied. Of chromosomal transcripts it is the labeling of polymerase II-promoted RNA that is interrupted preferentially, but 4–5S RNA is influenced as well. The labeling of hnRNA and 4–5S RNA is diminished by 70–90% and 45–60%, respectively, while the incorporation into the nucleolar preribosomal RNA remains essentially unchanged. Labeled adenosine is transported efficiently across the plasma membrane and becomes phosphorylated to AMP, ADP, and ATP, of which ATP predominates at noninhibitory concentrations. The rate of the formation of [^{3}H]AMP is, however, enhanced in response to the increase in external adenosine doses, whereas that of [^{3}H]ATP increases only slowly or remains essentially unaltered. A rise in exogenous [^{3}H]adenosine concentration to 200 μM yields a [^{3}H]ATP/[^{3}H]AMP ratio that is about one order of magnitude lower than that at 20 μM of the nucleoside. In parallel with this, there is a gradual repression of the labeling of chromosomal RNA. A similar treatment with guanosine produces only minor reduction in GTP/GMP quotient and does not influence significantly the labeling of any sizable RNA fraction. Thus the experimental data strongly indicate that the purine ribonucleoside adenosine, but not guanosine, gives rise to a markedly diminished triphosphate/monophosphate quotient simultaneously with a selective suppression of the labeling of chromosomal RNA, especially hnRNA, when applied in overdoses. The sequence of hnRNA events during inhibition by adenosine resembles the effect of the purine nucleoside analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, indicating that the site of inhibitory action is at or close to the initiation of transcription.

The interest in the purine ribonucleoside adenosine and its regulatory functions within a broad range of scientific fields, including cell biology, biochemistry, and medicine, has increased considerably during the last decade. Adenosine added to cells is transported across plasma membrane by a facilitated diffusion mechanism via carrier proteins and by free diffusion (for reviews, see references 3 and 37). It is efficiently utilized through the purine salvage pathways operating intracellularly. One main route of utilization is deamination by adenosine deaminase to inosine (34, 49), which in turn may become converted to hypoxanthine by purine nucleoside phosphorylase (28). Another one is phosphorylation by adenosine kinase to AMP (28, 34). It is to a large extent the balance between the activity of adenosine deaminase and adenosine kinase, which may vary in various cell types at different conditions, that determines the choice of the utilization route and the concomitant biological effects of adenosine (34, 36, 48). In addition, adenosine may be phosphorylyzed to adenine and ribose-1-P (51). It also appears evident, at least in certain cell systems, that adenosine is capable to influence cellular metabolism by binding to extracellular adenosine receptors without penetration of the plasma membrane (42, 6; for review, see reference 20).

Among biochemical effects of adenosine, the stimulation (or inhibition) of the accumulation of cAMP (5, 20, 32), the suppression of *de novo* pyrimidine synthesis in mammalian cells (26, 28), and the glucose-like restorative action on cellular energy balance with an elevation of the content of adenine nucleotides in lymphocytes (33) are the best known. A well-established biological effect of exogenous adenosine is its cy-

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totoxicity, leading to a growth arrest in mammalian cells (26, 27). In addition, it possesses a prominent influence on secretion of a great number of hormones, on regulation of blood flow (4, 35), and may even work as a transmitter in the peripheral nerve system (43). The toxicity of adenosine, which is enhanced in the presence of adenosine deaminase inhibitor, can to a certain extent be attributed to a block of pyrimidine synthesis (26, 28) or inhibition of immune response (1). There is, however, support for the view that additional factors are involved in the process of metabolic effects and cytotoxicity exerted by adenosine. An interference with ribosome formation and a reduced rate of preribosomal 45S RNA synthesis (24) as well as a nucleolar fragmentation (25, 40) have been reported previously. Adenosine added in overdoses to salivary gland cells of *Chironomus tentans* resulted in a considerable size reduction of the Balbiani rings (BRs), and the incorporation of [^{3}H]uridine into BR RNA fell considerably (2).

The present communication provides information on the biochemical effects of exogenous adenosine in explanted salivary gland cells of *Chironomus tentans* and on the intracellular fate of [^{3}H]adenosine. Given in doses of 60–1,600 μM, adenosine diminishes the rate of RNA labeling in a differentiated manner by a preferential interference with the incorporation of label into chromosomal RNA. The rate of the labeling of hnRNA and 4–5S RNA is reduced by 70–90% and 45–60%, respectively, while that of nucleolar preribosomal RNA remains essentially unaltered. [^{3}H]Adenosine is efficiently phosphorylated intracellularly even at overdoses but the [^{3}H]ATP/[^{3}H]AMP ratio decreases markedly with increasing [^{3}H]adenosine concentrations. The inhibitory properties of adenosine are similar, but not identical, to that of the purine nucleoside analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (9, 11, 12).

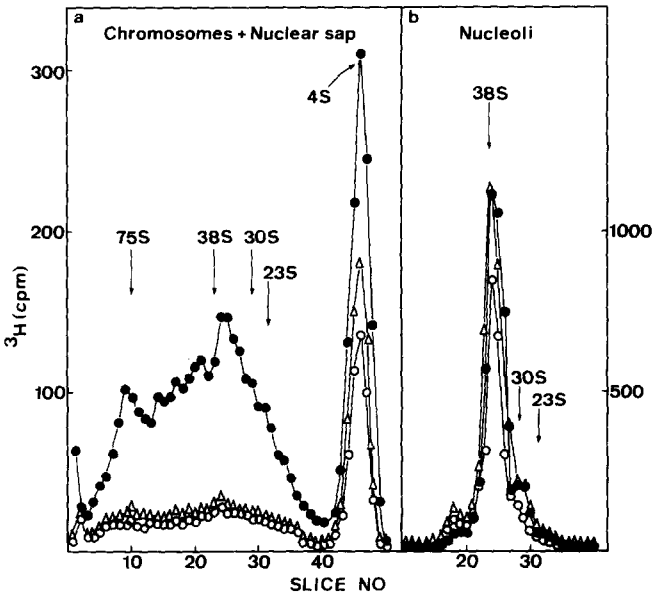

FIGURE 1 Electrophoretic analyses of chromosomal plus nuclear sap RNA (*a*) and nucleolar preribosomal RNA (*b*) labeled for 30 min with [^{3}H]uridine in the absence and the presence of adenosine after 30 min of preincubation. Five glands were incubated at 18°C for 30 min in 50 μl of incubation medium containing 200 μM of adenosine. They were then transferred to another 50 μl of the same medium containing adenosine, 200 μCi of uridine, and incubated for 30 min. For labeling with [^{3}H]uridine in the absence of adenosine, the sister glands were used in an otherwise parallel procedure. After incubation, the glands were fixed in 70% ethanol, and chromosomes plus nuclear sap and nucleoli were dissected out from four cells per gland. The labeled RNA from each sample was released by proteinase K-SDS treatment, and electrophoresis was conducted in 1% agarose gel slabs. The radioactivity was measured in a Packard liquid-scintillation spectrometer. *E. coli* RNA was used as marker. The position of 75S was determined in parallel analysis of BR RNA. The amount of label in chromosomal plus nuclear sap 4–5S and hnRNA decreased from 1,150 to 475 cpm, and from 3,090 to 610 cpm, respectively, while the labeling of nucleolar preribosomal RNA is reduced from 4,510 to 3,430 cpm (uncorrected values). For more details see Materials and Methods. (●) Normal cells; (○) adenosine-treated cells; (△) adenosine-treated cells corrected for change in labeling of the UTP pool.

MATERIALS AND METHODS

Salivary glands were isolated from fourth instar larvae of the dipteran *Chironomus tentans* (30). Glands were explanted into 50 or 100 μl of modified Cannon's medium (41) supplied with [^{3}H]uridine (44 Ci/mmol), [^{3}H]adenosine (20 Ci/mmol), or [^{3}H]guanosine (6.4 Ci/mmol) (The Radiochemical Centre, Amersham, England), and with unlabeled adenosine or guanosine (Sigma Chemical Co., St. Louis, Mo.). After incubation the cells were extracted three times for 20 min each with 100-μl volumes of 70% ethanol to release free nucleosides and nucleotides and for fixation of the glands at the same time (17). After fixation, nucleoli, chromosomes, and nuclear sap were isolated by microdissection. The pooled nucleolar and chromosomal samples, or unfractionated cells, were then digested with 100 μl of 20 mM Tris-HCl buffer, pH 7.4, containing 2 mM EDTA, 0.5% SDS (Serva, Heidelberg, W. Germany) and 0.1% proteinase K (Merck, Darmstadt, W. Germany) for 5 min at room temperature (18). After addition of 10 μl of 1 M NaCl, the RNA was precipitated with 2.5 vol of ethanol at −20°C overnight.

The aqueous-ethanol-soluble nucleosides and nucleotides were separated by thin-layer electrophoresis on Polygram Cel 300 plastic sheets (Machery-Nagel, Bureu, W. Germany), using 50 mM citrate buffer, pH 5.2, at 20 V/cm for ~1.5 h in a Desaga (Heidelberg, W. Germany) thin-layer electrophoresis apparatus (17).

The separation of RNA was carried out in 1% agarose (Serva, Heidelberg, W. Germany) gel slab, using 20 mM Tris-HCl buffer, pH 8, containing 20 mM NaCl, 2 mM EDTA, and 0.2% SDS, as previously described (30).

After the electrophoretic run, the plastic sheets, or gel slabs, were cut out and the slices were transferred to Packard scintillation vials, each containing toluene, soluene and Permablend III (Packard Instrument Co., Inc., Downers Grove, Ill.). The radioactivity was measured in a Packard (3380) liquid scintillation spectrometer at an efficiency of ~35% and at a background of 10–12 cpm.

RESULTS

Adenosine Suppresses Selectively the Labeling of Chromosomal and Nuclear Sap RNA

The electrophoretic profiles of chromosomal plus nuclear sap RNA (Fig. 1 *a*) and nucleolar RNA (Fig. 1 *b*) after a 30-min incubation of salivary glands with adenosine, followed by a labeling period with [^{3}H]uridine for 30 min in the continued presence of adenosine, are presented in Fig. 1. The control glands were subjected to the same type of treatment but in the absence of the purine nucleoside. The pattern of normal chromosomal plus nuclear sap RNA displays the usual bimodal distribution with a distinct peak in the 4–5S range (7, 11) and with label heterogeneously distributed in the 10–100S range, including two minor peaks at 35S and 75S RNA (15). The labeling pattern of nucleolar RNA labeled for 30 min is dominated by the preribosomal 38S RNA (41). As seen in Fig. 1, a treatment of glands with adenosine at 200 μM reduces the labeling of hnRNA and 4–5S RNA by ~80 and 60% (Fig. 1 *a*), respectively, while the incorporation into nucleolar RNA is diminished only by 24% (Fig. 1 *b*).

To examine to what extent the reduced rate of RNA labeling in the presence of adenosine might be an expression for a

reduction of the uptake of [^{3}H]uridine and thus a diminished labeling of the UTP pool, the ethanol-soluble nucleotides released from glands were analyzed by thin-layer electrophoresis. Fig. 2 shows the electrophoretic pattern of the labeled phosphorylated derivatives of [^{3}H]uridine including the precursor itself with and without treatment with adenosine. In addition to the expected UMP, UDP, and UTP, there is an unidentified prominent peak migrating somewhat faster than UMP. The result in Fig. 2 indicates that the addition of adenosine decreases the rate of phosphorylation reaction, including labeling of UTP pool, by ~26%. Whether or not also the velocity of the transport of uridine across the plasma membrane was affected cannot be evaluated on the basis of the present experimental design.

The data in Figs. 1 and 2 taken together strongly indicate that adenosine added to explanted salivary glands differentially inhibits the labeling of chromosomal RNA, especially hnRNA. The rate of hnRNA and 4–5S RNA production decreases by 75 and 48%, respectively, whereas the effect on synthesis of nucleolar 38S RNA is insignificant.

The dose response of adenosine on the labeling of nucleolar preribosomal RNA, and on the chromosomal plus nuclear sap hnRNA and 4–5S RNA is illustrated in Fig. 3. Explanted salivary glands were preincubated with adenosine at various concentrations for 30 min before labeling for 30 min with [^{3}H]uridine in the continued presence of adenosine. The control glands were labeled in similar conditions but without adenosine. After dissection of nucleoli and chromosomes plus nuclear sap, and electrophoresis of RNA released from these cell components, the sum of labeled material was calculated from the resulting gel profiles for nucleolar preribosomal RNA, and for the chromosomal plus nuclear sap 4–5S and 10–100S RNA. The values shown are corrected for reduction of labeling in UTP pools. Fig. 3 shows that the synthesis of preribosomal RNA is resistant to the action of adenosine within a broad concentration range (30–1,600 μM). The suppression of

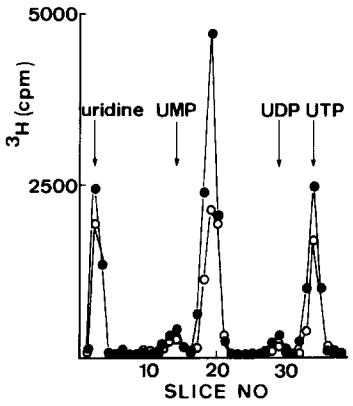

FIGURE 2 Electrophoretic analyses of ethanol-soluble cell extracts labeled for 30 min with [^{3}H]uridine in the absence and presence of adenosine after 30 min of preincubation. Five salivary glands from five different animals and their sister glands were incubated with and without 200 μM of adenosine, respectively, as described in the legend to Fig. 1. The cells were then extracted with 70% ethanol and 2% of the extracts were subjected to thin-layer electrophoresis on precoated plastic sheets. At the end of the run, the sheets were cut and the strips transferred to Packard scintillation vials. Uridine, UMP, UDP, and UTP were used as reference substances. The labeling of UTP decreased from 4,780 to 3,530 cpm. For other data see Materials and Methods. (●) Normal cells; (○) adenosine-treated cells.

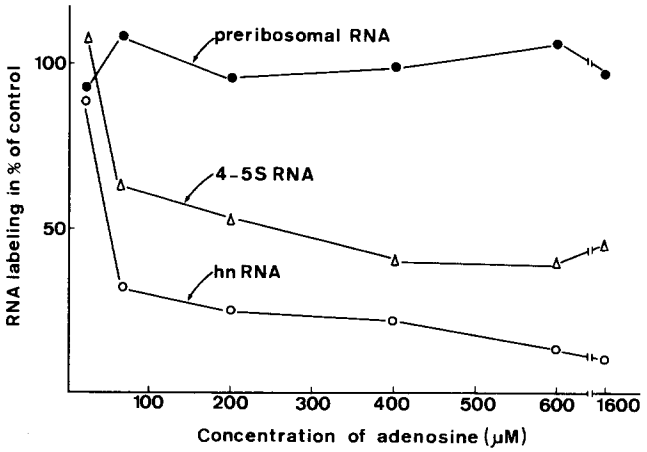

FIGURE 3 Effect of different doses of adenosine on labeling on hnRNA (10–100S RNA), 4–5S RNA, and nucleolar preribosomal RNA. Five glands from different animals were placed in an incubation droplet of 50 μl containing 30, 65, 200, 400, 600, or 1,600 μM of adenosine and were incubated at 18°C for 30 min. They were then transferred to another 50 μl of the same medium containing adenosine, and 100 μM of [^{3}H]uridine and incubated for 30 min. For labeling with [^{3}H]uridine in the absence of adenosine, the sister glands were used in an otherwise parallel procedure. After incubation the glands were fixed and the ethanol-soluble radioactivity was analyzed by thin-layer electrophoresis. Chromosomes plus nuclear sap and nucleoli were isolated from four cells per gland by microdissection. After electrophoresis in 1% agarose gel, the radioactivities were computed from the resulting gel profiles for nucleolar preribosomal RNA (●), chromosomal plus nuclear sap hnRNA (10–100S RNA) (○), and 4–5S RNA (△), respectively. The values are corrected for reduction of label (0–25%) in the UTP pool. For more details see the legends to Figs. 1 and 2.

hnRNA and 4–5S RNA labeling is largely dose dependent in the 30–100 μM range and the degree of inhibition increases only moderately at higher concentrations of adenosine. It is also evident from Fig. 3 that the extent of inhibition of hnRNA synthesis exceeds that of the 4–5S RNA fraction at all investigated doses. However, the labeling of an hnRNA fraction is apparently resistant to the adenosine-induced suppression.

Intracellular Fate of Adenosine Applied at Low and High Concentrations

To examine the fate of adenosine in salivary gland cells at noninhibitory and inhibitory concentrations, explanted glands were incubated with 20 and 200 μM, respectively, of [^{3}H]-adenosine for 60 min. The ethanol-soluble cell extracts were then analyzed by thin-layer electrophoresis (Fig. 4). As expected, the electrophoretic patterns reveal the presence of three phosphorylated adenosine metabolites, AMP, ADP, and ATP, in addition to the unmetabolized precursor. The 10-fold increase in exogenous adenosine concentration gave rise to an about 15.7-fold gain in [^{3}H]AMP content, but the rate of [^{3}H]-ATP accumulation increased only by about 1.6 times. Accordingly, the quotient [^{3}H]ATP/[^{3}H]AMP, 2.4, observed at low dose treatment, decreased by about one order of magnitude to 0.23 in glands treated with 200 μM adenosine.

The incorporation of [^{3}H]adenosine into total RNA was studied by electrophoresis in a 1% agarose gel after incubation for 60 (Fig. 5*a*) and 150 min (Fig. 5*b*). Fig. 5 shows the radioactivity patterns of RNAs after labeling with 20 and 200 μM of [^{3}H]adenosine. The distribution of RNA from cells

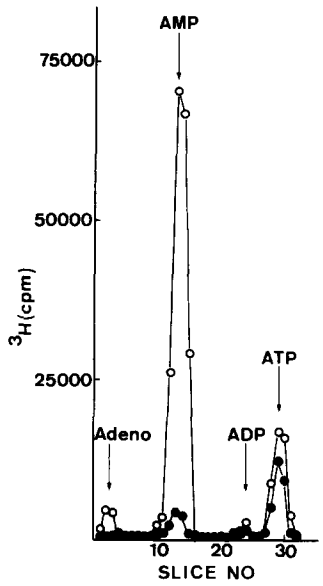

FIGURE 4 Electrophoretic analyses of ethanol-soluble cell extracts labeled for 60 min in the presence of 20 and 200 μM of [^{3}H]-adenosine. Three glands from three different animals were incubated in 50 μl of medium containing 20 μM of [^{3}H]adenosine and their sister glands were placed in another 50 μl of the same medium containing 200 μM of [^{3}H]adenosine. The glands were incubated at 18°C for 60 min. After incubation the glands were extracted with 70% ethanol, and 2% of the extracts were exposed to thin-layer electrophoresis. Adenosine, AMP, ADP, and ATP were used as marker substances. The labeling of AMP and ATP increased from 12,630 to 198,860 cpm and from 28,800 to 47,000 cpm, respectively. For more details see Materials and Methods. (●) 20 μM of [^{3}H]adenosine; (○) 200 μM of [^{3}H]adenosine.

treated with 200 μM adenosine is corrected for the gain in incorporation of label into the ATP pool by ~60%. The electrophoretic profiles exhibit the distribution of previously established peaks at 4–5S, at 23S, 30S, and 38S, constituting the nucleolar preribosomal RNA components (41), and at 75S, including BR RNA (16, 29). The heterogeneous background labeling in the 10–100S region, as well as BR RNA, consists to a large extent of hnRNA that is sensitive to α-amanitin (10) or DRB (9, 14). As seen in Fig. 5, the incubation with [^{3}H]-adenosine at the high dose led to a differential inhibition of RNA synthesis in line with the results presented in Fig. 1, although the degree of inhibition is lower in Fig. 5 than in Fig. 1. The advantage with this type of experimental design lies in that adenosine can serve both as inhibitor and exogenous RNA precursor and that the difficulties associated with the determination of the effect of adenosine on uridine uptake are thereby largely eliminated. The lower inhibitory effect in Fig. 5 might, at least partly, be explained by the fact that no preincubation with unlabeled adenosine preceded the incubation with labeled adenosine.

There is a Resemblance between Adenosine and DRB as to the Intracellular as well as the Molecular Site of Inhibitory Action

When salivary glands were incubated as described in Fig. 5 *a* for 60 min but the high adenosine dose was replaced by 65 μM DRB (both control and treated glands were labeled with 20 μM [^{3}H]adenosine) and the total RNA was analyzed, the electrophoretic pattern revealed a similar differential inhibition of hnRNA labeling as seen in Fig. 5 (Fig. 6). The inhibition of hnRNA, especially 75S RNA, with this type of experimental design is only partial, but if glands are pretreated with DRB before addition of isotopic RNA precursor the suppression of hnRNA synthesis is almost complete (11, 14). A comparison of the results in Figs. 5 and 6 indicates that both adenosine and DRB exert a preferential inhibitory action on labeling of hnRNA when used at appropriate doses. It is, however, not possible to conclude on the basis of these data whether adenosine like DRB interferes with transcription events at or close to the site of initiation (11, 13, 14, 44). This question was further studied by analyses of RNA derived from microdissected samples of chromosomes I to III. The rationale for this experiment was previous studies, which provided evidence to support the nascent character of chromosomal hnRNA, at least of BR RNA (13), and the fact that the administration of an inhibitor of initiation to cells allows preinitiated growing chains to be completed while reinitiation is blocked. Thus, there is a preferential inhibition of the labeling in the lower molecular weight regions of hnRNA spectrum (11–13). If, however, the time of incubation with an inhibitor of transcription initiation is sufficiently long, labeling of small as well as large hnRNA transcripts is equally eliminated. Salivary glands were incubated with [^{3}H]uridine for 60 min in the presence of unlabeled adenosine. Another group of glands was labeled for 30 min in the absence, followed by 30 min in the presence, of adenosine.

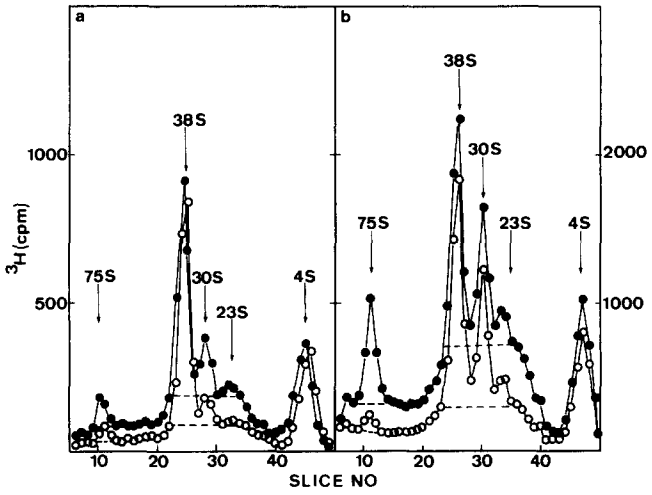

FIGURE 5 Electrophoretic analyses of the total cellular RNA after labeling in the presence of 20 and 200 μM of [^{3}H]adenosine for 60 min (*a*) and 150 min (*b*). Three salivary glands from three different animals were incubated in 50 μl of medium containing 20 μM of [^{3}H]adenosine and their sister glands were placed in another 50 μl of the same medium containing 200 μM of [^{3}H]adenosine. The glands were incubated at 18°C for 60 min. After fixation, the RNA was released by proteinase K-SDS treatment and subsequently separated by electrophoresis in a 1% agarose gel slab. The pattern of RNA derived from glands incubated with 200 μM of [^{3}H]adenosine is corrected for the increase in labeling in the ATP pool by ~60%. The counts above the dashed lines in the 23S, 30S, and 38S peaks and in the 75S peak were considered to be nucleolar preribosomal RNA and BR RNA, respectively. The labeling of 4–5S RNA, hnRNA, and 75S RNA decreased from 1,440; 5,280; and 200 cpm to 1,240; 2,510; and 100 cpm, respectively in *a* and from 2,870; 21,360; and 1,570; to 2,240; 8,980; and 240 cpm, respectively in *b*. The radioactivity in preribosomal RNA was diminished from 2,125 to 2,070 cpm in *a* and gained from 3,400 to 3,570 cpm in *b*. For more details see Materials and Methods (●) 20 μM of [^{3}H]adenosine; (○) 200 μM of [^{3}H]adenosine.

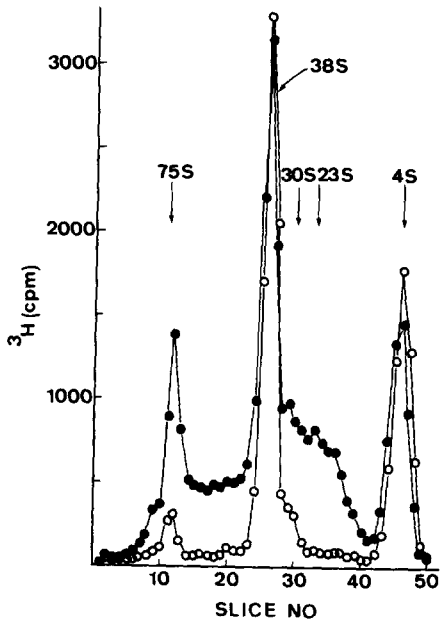

FIGURE 6 Electrophoretic analyses of the total cellular RNA after labeling with [^{3}H]adenosine in the presence and absence of DRB. Five salivary glands from five different animals were incubated in 50 μl of medium containing 20 μM of [^{3}H]adenosine and 65 μM of DRB for 60 min at 18°C. For labeling in the absence of DRB, the sister glands were used in parallel. For other data see the legend to Fig. 5 and Materials and Methods. (●) Normal cells; (○) DRB-treated cells.

The control glands were labeled for 60 min in the absence of adenosine (Fig. 7 *a*). In another similar labeling experiment adenosine was replaced by DRB (Fig. 7 *b*). The electrophoretic pattern of chromosomal RNA in the 10–100S range after 30 min of adenosine treatment displays a shift in the radioactivity distribution towards the higher molecular weight regions. The labeling of short transcripts is preferentially suppressed and the material migrating as 75–100S is not appreciably influenced. The degree of decrease of radioactivity in hnRNA seems to be inversely related to the size of RNA molecules. However, when adenosine and [^{3}H]uridine are added simultaneously, and the inhibitor is present during the whole period of incubation, the labeling of RNA decreases markedly in the entire size spectrum of hnRNA. The incorporation of label into short and long chains is inhibited approximately to the same extent. Thus adenosine possesses a differential and time-dependent inhibitory action on labeling of chromosomal hnRNA. A comparison of the effect of adenosine with that of DRB with respect to the inhibitory action on labeling of hnRNA, as reflected by the differential and time-dependent inhibition of nascent chains, reveals obvious similarities between the two (Fig. 7 and references 11 and 12). The extent of inhibition with adenosine is, however, lower than with DRB, but the relative electrophoretic distribution of the drug-resistant hnRNA is roughly the same. The experimental data in Figs. 5–7 give a strong support to the contention that the intracellular and molecular sites of the inhibitory action for the purine nucleoside analogue DRB and that for the natural purine nucleoside adenosine are probably closely related.

Guanosine is without Measurable Effect on Labeling of RNA with [^{3}H]Uridine

Salivary glands were incubated for 30 min in the presence of 200 μM guanosine followed by incubation for another 30 min with [^{3}H]uridine in the continued presence of guanosine. The control glands were subjected to the same type of treatment but in the absence of guanosine. The electrophoretic profile of the total salivary gland RNA derived from guanosine-treated cells, depicted in Fig. 8, does not differ significantly from that of the control material. The result strongly suggests that guanosine, unlike adenosine, is unable to influence to an appreciable degree the synthesis of any sizable RNA fraction even when applied in overdoses. The analysis of the ethanol-soluble radioactivity released from guanosine-treated glands shown in Fig. 8 reveals only a minor reduction (~20%) in labeling of UTP pool (data not shown).

Intracellular Fate of Guanosine Administered at Low and High Concentrations

In view of the fact that guanosine is without measurable effect on RNA synthesis, it was of interest to examine the

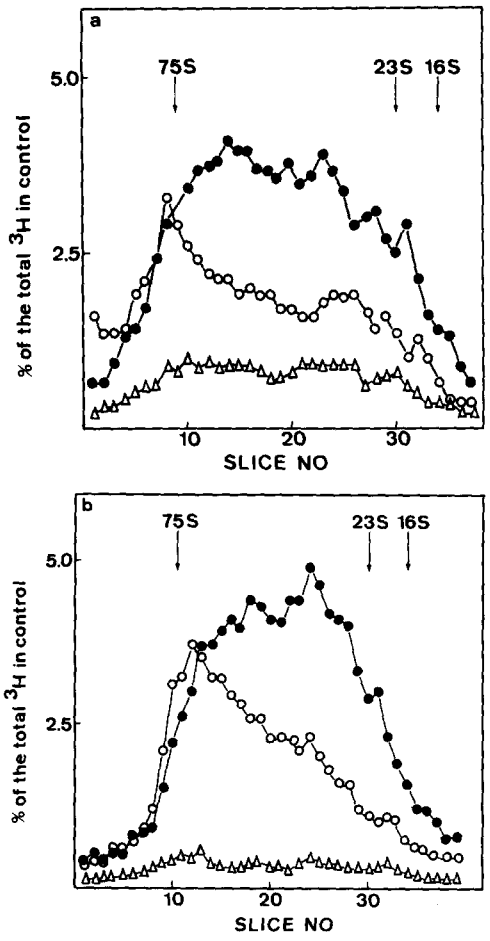

FIGURE 7 Electrophoretic analyses of chromosome I to III RNA after labeling in the absence and presence of adenosine (a) and DRB (*b*). Five glands from five different animals were incubated in 50 μl of medium containing 100 μCi of [^{3}H]uridine and 200 μM of adenosine or 65 μM of DRB and were incubated at 18°C for 60 min; in the presence of inhibitor during the whole incubation period, 30 min in the absence, followed by 30 min in the presence, of inhibitor, and in the absence of inhibitor during the whole period. For labeling in the presence of inhibitor for 30 min (○) and in the absence of inhibitor (●), sister glands were used. Chromosomes I to III were dissected from 25 cells (five glands). For other data, see the legend to Fig. 5 and Materials and Methods. (●) Labeling in the absence of inhibitor; (○) labeling in the presence of inhibitor for 30 min; (△) labeling in the presence of inhibitor for 60 min.

extent of intracellular phosphorylation of [^{3}H]guanosine administered at low and high concentrations. Two groups of salivary glands (sister glands) were incubated with 23 and 230 μM of [^{3}H]guanosine for 30 min and the ethanol-soluble material was separated by thin-layer electrophoresis. Fig. 9 shows that the rate of [^{3}H]GDP and [^{3}H]GTP formation remains essentially unaltered despite a 10-fold enhancement of the exogenous guanosine concentration. The intracellular concentration of unphosphorylated [^{3}H]guanosine increased, however, in response to the enhanced cellular dose. The [^{3}H]GTP/[^{3}H]GMP ratio exhibited only a moderate decrease, by ~40%, at the higher guanosine concentration. This is in sharp contrast to the [^{3}H]ATP/[^{3}H]AMP quotient, observed in similar experiments with low and high doses of [^{3}H]adenosine, which is one order of magnitude higher at the enhanced (inhibitory) nucleoside concentration (Fig. 4).

DISCUSSION

Effect of Adenosine on Labeling of Chromosomal RNA Probably Reflects Transcription Inhibition

The ribonucleoside adenosine added at higher doses exerts a novel inhibitory effect on labeling of chromosomal RNA, particularly the polymerase II-promoted hnRNA, but leaves the labeling of nucleolar preribosomal RNA unaltered. Radioactive adenosine applied at inhibitory concentrations is efficiently metabolized intracellularly to AMP. The AMP metabolite formed is, however, only to a limited extent converted to di- and triphosphate derivatives, and labeled AMP predominates over other labeled adenosine metabolites. The determination of the exact extent of RNA synthesis by using exogenous nucleosides as RNA precursors to measure incorporation of label into RNA, and to express inhibition or stimulation of the rate of RNA formation, is always beset with difficulties. One major problem arises from possible changes in precursor uptake caused by interference with the transport step and/or with phosphorylation of the exogenous precursor. In the present studies the incorporation of label into the UTP pool and RNA was determined simultaneously in both control and adenosine-treated cells. Hence the incorporation of label into RNA could roughly be corrected for alterations caused by adenosine in the labeling of UTP (or ATP) pool by interference with the phosphorylation reaction. Another problem that may complicate the assessment of RNA synthesis as estimated by incorporation of exogenous precursors stems from the possibility that intracellular nucleotide pools are compartmented. That is, RNA is manufactured from precursor pools that are not in equilibrium with the main internal nucleotide pools. Alternatively, the formation of nucleolar and chromosomal RNA fractions is fed from separate pools. If so, the differential suppression of labeling of chromosomal RNA by adenosine might be attributed to a selective effect on labeling of nucleotide precursor pools rather than on RNA synthesis on the chromosomes.

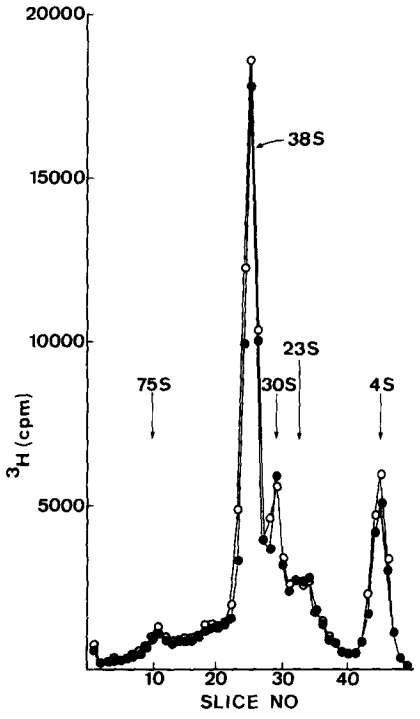

FIGURE 8 Electrophoretic analyses of the total cellular RNA labeled for 30 min with [^{3}H]uridine in the absence and presence of guanosine after 30 min of preincubation. Five salivary glands from 5 different animals were incubated in 50 μl of medium containing 200 μM of guanosine for 30 min at 18°C. They were then transferred to another 50 μl of the same medium containing guanosine and 200 μCi of [^{3}H]uridine, and incubated for 30 min. After incubation and fixation in 70% ethanol, the RNA was extracted and analyzed in a 1% agarose gel. For more details see the legend to Fig. 1 and Materials and Methods. (●) Normal cells; (○) guanosine-treated cells.

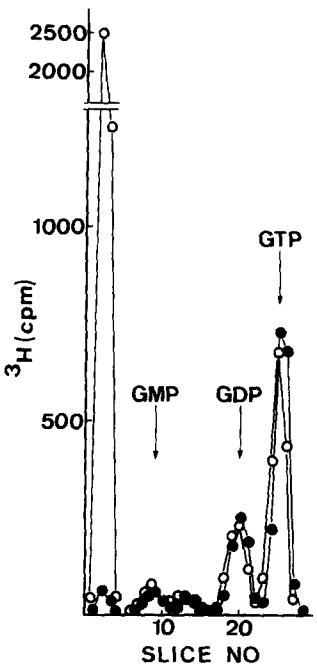

FIGURE 9 Electrophoretic analysis of ethanol-soluble cell extracts labeled for 30 min in the presence of 23 and 230 μM [^{3}H]guanosine. Three salivary glands from three different animals were incubated in 50 μl of medium containing 23 μM of [^{3}H]guanosine and their sister glands were placed in another 50 μl of the same medium containing 230 μM of [^{3}H]guanosine. The glands were incubated for 30 min at 18°C. Guanosine, GMP, GDP, and GTP were used as marker substances. For other data see the legend to Fig. 4 and Materials and Methods. The amount of unphosphorylated isotope increased from 115 to 3,500 cpm, labeled GMP from 190 to 260 cpm, and labeled GTP decreased from 1,740 to 1,690 cpm. (●) 23 μM of [^{3}H]guanosine; (○) 230 μM of [^{3}H]guanosine.

The existence of compartmented nucleotide pools, of which some are rapidly draining nuclear pools, constituting the immediate precursor for RNA synthesis while others are slowly draining cytoplasmic pools, were supported in previous studies (38, 39). If adenosine would block the flow of labeled UTP selectively into this tentative chromosomal UTP pool, then the diminished RNA labeling might reflect a pool effect rather than inhibition of the rate of RNA synthesis. This possibility seems, however, unlikely in view of evidence presented against the existence of separate pools from which nucleolar preribosomal and nucleoplasmic hnRNA might be derived (45, 50). In addition, when adenosine is utilized both as inhibitor and as labeled RNA precursor, the incorporation of radioactivity into hnRNA is blocked to a large extent, while the labeling of rRNA remained unchanged (Fig. 5).

Adenosine, Like DRB, is a Likely Inhibitor of Some Early Event(s) in Transcription of hnRNA Genes by Polymerase II

The transcripts affected by adenosine toxicity exhibit α-amanitin sensitivity consistent with their being polymerase II products, unlike 4–5S RNA or ribosomal RNA (10, 12, 31). This polymerase seems especially susceptible to adenosine and DRB. In view of the chemical characteristics of DRB, it is a highly remarkable fact that the naturally occurring purine nucleoside adenosine and this nucleoside analogue may induce so closely related effects on RNA synthesis, the more so as the nonshugar portion of DRB deviates substantially from that of adenosine. There is a 5,6-dichlorobenzimidazole in DRB versus a 6-aminopurine in adenosine. As yet, it is not possible to decide whether adenosine and DRB exert their inhibitory activity by identical molecular mechanisms.

As is shown in Fig. 7, there is a time-dependent, differential suppression of growing hnRNA chains with a preferential suppression of shorter chains. Thus the present type of experiments indicates that the formation of a majority of hnRNA molecules is interrupted by adenosine at or near the site of initiation of growing chains. Whether adenosine or DRB (13) inhibits transcription initiation per se in *Chironomus* cells or promotes early chain termination similar to that found in HeLa cells (46, 47) and in adenovirus-infected HeLa cells (22, 23) is a mechanistic question that cannot yet be answered. It should be pointed out in this connection that, contrary to the data in *Chironomus* (17, 19), no measurable intracellular phosphorylation of DRB was observed in Ehrlich ascites cells (8).

There are Differences between Adenosine and DRB in Intracellular Phosphorylation and in Selectivity and Efficiency of Inhibition

Even though adenosine, and also DRB, is transported across the plasma membrane into the cells, the intracellular fate of the two is rather different. Adenosine is metabolized to mono-, di-, and triphosphates (Fig. 4), while DRB is phosphorylated only to monophosphate metabolites (17, 19). It is to be noted, however, that the predominating adenosine derivative obtained at inhibitory concentration is AMP, although at low (noninhibitory) doses it is ATP that is the most abundant adenosine metabolite. Whether the anomalous AMP accumulation at higher doses is involved in the inhibitory effect of adenosine on RNA synthesis and whether the pattern of inhibition in *Chironomus* mimics that in mammalian cells is a subject for further investigation.

Adenosine differs from DRB in the degree of selectivity and of efficiency in the inhibitory action on hnRNA synthesis. Adenosine is a less selective inhibitor than DRB because it diminishes the labeling of 4–5S RNA as well and, in addition, ~20–40% of hnRNA synthesis is adenosine resistant. By contrast, it is only a minute fraction, <10%, of hnRNA synthesis that survives DRB treatment, and there is no appreciable effect on the formation of 4–5S RNA (11, 14).

The best-established biochemical effect of adenosine is exerted through cell surface receptors and the adenylate cyclase system (20). There are, however, good reasons for believing that this nucleoside possesses a broad range of effects on cellular metabolism and regulation (21). Thus, the search for possible mechanisms responsible for the unexpected effect described here of the ribonucleoside adenosine, but not of guanosine, on the expression of chromosomal genes must be directed towards a number of alternative approaches, including the investigation of the exceptional AMP accumulation.

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