Theophylline Incorporation into the Nucleic Acids of Theophylline-stimulated Melanoma Cells

MARK L. STEINBERG, PH.D., AND J. R. WHITTAKER, PH.D.
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, U.S.A.

Theophylline, an inhibitor of cAMP-degrading phosphodiesterase, stimulates melanin biosynthesis in cultures of RPMI 3460 hamster melanoma cells. Although theophylline does produce an initial transient elevation of intracellular cAMP levels, long-term treatment with theophylline produces a significant decrease in cAMP content. There is an inhibition of the theophylline stimulation by dibutyryl-cAMP; this is apparently caused by interference of dibutyryl-cAMP with the uptake and incorporation of theophylline, as shown by experiments with 3H-theophylline. An alternative theory is that theophylline, being a methylxanthine compound, is metabolized by the cell and possibly causes melanotic stimulation by becoming incorporated into cellular nucleic acids or by altering the normal nucleic acid metabolism. The following observations are consistent with this theory:

1. 3H-theophylline was incorporated into both trichloroacetic acid (TCA)-soluble and TCA-insoluble cell fractions; most of the insoluble label became soluble after digestion with ribonuclease and deoxyribonuclease. (2)
2. These nuclease digests of the 3H-theophylline-labeled TCA-insoluble cell fractions contained 3H-labeled material that chromatographed differently from normal nucleotides on ion exchange thin layer sheets. (3) The acid-soluble pool of 3H label disappeared rapidly while both the insoluble label and the inunction of melanogenesis remained stable for 50 hr after the removal of exogenous 3H-theophylline.

Cyclic AMP is an effector of phenotypic expression in a wide variety of cell types in tissue culture including sarcoma cells [1,2], muscle cells [3,4], chick retinal epithelial cells [5], and melanoma cells [6-9]. Much of the evidence which implicates this nucleotide as a causal agent in these effects has come from studies using compounds known to inhibit the action of cAMP-degrading phosphodiesterases. The structures of many of these inhibitors closely resemble those of purine bases. Although purine, pyrimidine and nucleoside analogs are known to affect nucleic acid metabolism by various mechanisms including substitution for normally occurring constituents of nucleic acids [10], the possibility that these drugs might exert phenotypic effects by a direct effect on nucleic acids has not been thoroughly studied. In a previous paper [11], we reported that the effects of the methylxanthine compound, theophylline, as a stimulator of melanotic phenotype in a line of Syrian hamster melanoma cells appeared to be distinct from its properties as a phosphodiesterase inhibitor.

To extend our earlier observations we have investigated the relationship between cAMP levels and induced melanogenesis in theophylline-treated cells. We have also investigated the alternative hypothesis that the melanotic effects of theophylline might be related to its uptake and metabolism by melanoma cells. Our results suggest that the melanotic effects of theophylline may be related to the agent's participation in nucleic metabolism rather than its role as an inhibitor of phosphodiesterase.

MATERIALS AND METHODS

Cell Culture

RPMI 3460, a permanent line of Syrian golden hamster melanoma cells [12], was obtained from Dr. Richard Davidson of Harvard University. Cells were grown at 37°C in Eagle's Basal Medium (Auto Pow BME; Flow Laboratories) containing 10% fetal calf serum (Flow) in an atmosphere of 5% CO2. Cultures were maintained in sealed 250 ml Falcon plastic culture flasks. In some cases cells were grown in stoppered 500 ml Erlenmeyer flasks (suspension cultures) on a rotating platform to maximize cell yield. Cultures of this type do not differ in any important way from those maintained in plastic culture flasks.

Labeling Conditions and Determination of Radioactivity

2.2-13C-thioucaril and 8-3H-theophylline were purchased from American/Searle Corp. 14C-labeled adenosine, cytidine, guanosine and uridine and their deoxy-counterparts were purchased from New England Nuclear Corp. 2.2-13C thiouracil was always present at a concentration of 1.0 mM and a specific activity of 0.5 mCi/mmol. The incorporation of this compound into trichloroacetic acid (TCA)-insoluble cell fractions was used as a measure of melanogenesis as described in detail by Whittaker [13]. When cultures were to be incubated in the presence of labeled theophylline or 2-thioucaril, detached cells were removed from the old culture medium by centrifugation, suspended in fresh radioactive medium and returned to the remaining monolayer cells. Concentrated 14C-labeled nucleosides were added directly to cultures (10 µl/culture). When TCA-soluble radioactivity was to be measured, harvested cells were washed twice in phosphate-buffered saline and resuspended in 0.7-2.0 ml of cold 10% TCA. After 1 hr at 4°C the insoluble material was pelleted and 0.5 ml aliquots of the supernatant were counted in 5 ml of Aquasol (New England Nuclear Corp.). Radioactivity and protein content of the insoluble fraction were determined as previously described [11].

cAMP Assay

Cell pellets obtained from cell harvests were frozen in an ethanol-Dry Ice mixture and then suspended in 0.7 ml of cold 0.2 N HCl in ethanol using a glass rod to break up the pellet. This extraction mixture (at 4°C) was stirred occasionally for 3 hr, after which suspended material was sedimented and the supernatant removed. The sedimented material was again suspended in 0.7 ml of cold 0.2 HCl in ethanol, centrifuged, and the supernatants combined. The supernatant fraction was lyophilized to dryness over P2O5 and the residue dissolved in an appropriate volume of assay buffer (4 mM EDTA, 0.05 M tris-HCl, pH 7.5) and assayed for cAMP as described by Gilman [14], except that unbound cAMP was adsorbed from reaction mixtures with charcoal. cAMP binding protein, 8-3H-cAMP standards and charcoal adsorbent were purchased from Amer sham/Searle Corp. in kit form. Known amounts of cAMP were added both to cell pellets after cell harvest and to blank tubes as internal standards. Recovery of standards was consistently greater than 90%.
Nuclease Assay

Harvested cells were pelleted by centrifugation, frozen, resuspended in cold 10% TCA and allowed to stand at 4°C for 1 hr. Suspended material was sedimented, the supernatant was discarded and the pelletted material washed: once in acetone, once in acetone-ether (1:1) and once in ether. The air-dried residue was homogenized in standard nuclease assay buffer (15 mM KCl in 0.02 M tris-HCl, pH 7.4). Aliquots of this suspension were removed for each reaction mixture. For DNase digestion each aliquot was combined with an equal volume of assay buffer containing 6 mM MgCl2, 2 mg/ml heparin and 450 units/ml of DNase (purified; Worthington Biochemicals). For RNase digestion each aliquot was combined with an equal volume of assay buffer containing 2 mM EDTA and 45 units/ml of RNase (Worthington Biochemicals). Reaction mixtures were incubated at room temperature and at various times 25 µl aliquots of each reaction mixture were removed and combined with 25 µl of bovine serum albumin solution (2 mg/ml) and 50 µl of cold 10% TCA. After 30 min at 4°C each sample was centrifuged and 80 µl of the supernatant was assayed for radioactivity in 5 ml of Aquasol. After 90 min it was determined that nuclease digestion was complete and reaction mixtures were precipitated by the addition of 1 volume of cold 20% perchloric acid (PCA). Radioactivity and protein content in the insoluble material were determined [11].

Ion Exchange Thin-Layer Chromatography

RNase digests. PCA-soluble fractions of RNase digests were neutralized with 10 n KOH. Precipitated KClO4 was removed by centrifugation and additional 10 n KOH was added to the supernatant to bring the final concentration of KOH to 0.3 N. The alkaline digests were incubated at 37°C for 15 hr and then neutralized with concentrated HCl and lyophilized to dryness. The residues were redissoved in 50 µl of H2O and applied to 20 x 20 cm polyethyleneimine (PEI) cellulose-coated thin-layer sheets (Baker Co.) along with 50–75 µg each of AMP, CMP, GMP, and UMP (present as mixtures of 2' and 3' nucleotides) as standards.

DNase Digests. PCA-soluble fractions of DNase digests were neutralized with 10 n KOH. Precipitated KClO4 was removed by centrifugation and the supernatant was lyophilized to dryness. The resulting residues were dissolved in 1 ml of 0.01 M Tris-HCl (pH 8.9) containing 3 units of snake venom phosphodiesterase (Worthington Biochemicals). After 16 hr at 37°C 1 volume of cold 20% PCA was added and the acid-soluble fractions were lyophilized to dryness after neutralization with 10 n KOH as before. These residues were redissolved in 50 µl of H2O and applied to PEI thin-layer sheets along with 50–75 µg each of dAMP, dCMP, dGMP and TMP (5'nucleotides).

Chromatography. Thin-layer sheets were chromatographed along the first dimension with 0.5 M formate buffer (sodium formate-formic acid, pH 3.5) for 2 min, then for 6 min in 1.0 M formate buffer and finally in 2.0 M formate buffer until the solvent front was within 1 cm of the edge of the sheet. Each sheet was washed in methanol for 7 min with agitation, air-dried and then chromatographed along the second dimension with formate buffer at pH 4.4 as before. Each sheet was again washed in methanol, air-dried, and spots were observed under a short wave UV lamp and circled in pencil. Sheets were cut into 0.5 cm squares as indicated (Fig 4) and each square was assayed for radioactivity in 5 ml of Aquasol. The procedure is discussed in more detail by Neuhaard, Randerath, and Randerath [15].

RESULTS

cAMP levels and Melanogenesis in Theophylline-Treated Cells

In untreated cell cultures cellular levels of cAMP gradually increased as cultures attained higher cell densities (Fig 1a and b). In these cultures the continuous increase in melanogenesis with time bore a direct relationship to cAMP levels (Fig 1a and c) as has been reported by others for mouse melanoma cells [16]. Although theophylline is known to inhibit phosphodiesterase, exposure of melanoma cells to theophylline for 36 hr caused only a transient elevation of cAMP levels; this modest stimulation (about 33% over controls at 0.75 mM theophylline) occurred during the first 24 hr of treatment. Longer periods of exposure to theophylline resulted in cAMP levels lower than those of control cultures. By contrast, melanogenesis was continuously stimulated above control values in cells exposed to theophylline over the entire 36 hr treatment period. Essentially the same results have been obtained in 2 other experiments in which the presence of theophylline caused a depression in cAMP levels between 12 and 48 hr following drug exposure.

It is possible that the theophylline-stimulated rise in melanotic activity might be caused by the initial elevation of cAMP levels that is observed during the first 12 hr of drug treatment. In the experiment shown in Table I the melanotic activity of 3460 cells was measured 24 hr after an initial 12 hr exposure to theophylline. The results of these experiments indicate that the 12 hr period of drug treatment could account for only 6–7% of the total stimulation in melanogenesis produced in cells exposed to theophylline over the entire 36 hr treatment period.

Fig 1. cAMP levels and 2-2,4-(C)thiouracil incorporation in theophylline-treated cells over time. Three series of cultures were set up on the basis of whether cells were grown in standard culture medium without additives (●—●), in the presence of 0.75 mM theophylline (□—□) or in the presence of 1.5 mM theophylline (○—○). (a) At the indicated times cultures were harvested, frozen and assayed for cAMP content. Each value represents the average of duplicate assays on 3 cultures. (b) Average cell number of the cultures harvested in (a). (c) Two hr before cultures were removed for cAMP assay the medium in 4 cultures from each series was changed to fresh medium containing 1.0 mM 2-2,4-(C)thiouracil. After 4 hr at 37°C these cultures were harvested and assayed for 2-thiouracil incorporation. A. absicae values represent the midpoints of periods of 2-thiouracil incorporation.
Effect of dbcAMP on Theophylline-Induced Melanogenesis

Although RPMI 3460 cells grown in the presence of theophylline show increases in melanin content and in rates of melanogenesis [11], exposure of theophylline-treated cells to dbcAMP caused a 43% reduction in the additional TCA-insoluble 2-thioracil incorporation brought about by 1.5 mM theophylline (Table II). This finding is apparently not related to drug-induced changes in thioracil uptake; the reduction in TCA-insoluble \(^{13}C\) incorporation brought about by dbcAMP is actually accompanied by an increase in the entry of 2-thioracil into the acid-soluble pool.

Effect of dbcAMP on Incorporation of \(^3H\)-Theophylline

dbcAMP reduces both the magnitude of the induction of melanogenesis by theophylline (Table II) and the uptake of theophylline by cells (Table III). Exposure to dbcAMP for 24 hr led to a decrease in \(^3H\)-theophylline label incorporated into both acid-soluble (an average reduction of 58%) and acid-insoluble (an average reduction of 51%) fractions.

### Table I. Effect of an initial 12-hr exposure to theophylline on melanotic activity measured at 36 hr culture time

<table>
<thead>
<tr>
<th></th>
<th>0-36 hr</th>
<th>1.5 mM theophylline</th>
<th>0-12 hr</th>
<th>1.5 mM theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control medium</td>
<td>1.36</td>
<td>1.59</td>
<td>4.92</td>
<td></td>
</tr>
<tr>
<td>Expt. I</td>
<td>1.44</td>
<td>1.74</td>
<td>5.80</td>
<td></td>
</tr>
</tbody>
</table>

* Suspension cell cultures were grown up to 36 hr in theophylline; 1 group had the theophylline medium replaced at 12 hr with normal control medium. At 36 hr all of the cultures were resuspended in control culture medium containing 1.0 mM 2-2-\(^{13}C\)-thioracil, and incubated 4 additional hr. Each value represents the average of 6 replicate cultures.

### Table II. Effect of theophylline and dbcAMP on incorporation of 2-2-\(^{13}C\)-thioracil into the acid-soluble fraction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acid-soluble</th>
<th>Acid-insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.49</td>
<td>15.36</td>
</tr>
<tr>
<td>1.5 mM theophylline</td>
<td>4.31</td>
<td>56.06</td>
</tr>
<tr>
<td>1.5 mM theophylline + 1.5 mm dbcAMP</td>
<td>5.09</td>
<td>32.03</td>
</tr>
</tbody>
</table>

* Suspension cell cultures were grown for 48 hr in the presence of the indicated agents. After this time the experimental medium was discarded and all cultures were incubated for an additional 4 hr in fresh culture medium containing 1.0 mM 2-2-\(^{13}C\)-thioracil. Each value represents the average of 4 replicate cultures.

### Table III. Effect of dbcAMP on incorporation of \(^3H\)-theophylline into acid-soluble and acid-insoluble cell fractions

<table>
<thead>
<tr>
<th></th>
<th>1.5 mM theophylline</th>
<th>1.5 mM dbcAMP + 1.5 mM theophylline</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. I</td>
<td>Acid-soluble</td>
<td>4.01</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble</td>
<td>3.81</td>
<td>54.3</td>
</tr>
<tr>
<td>Expt. II</td>
<td>Acid-soluble</td>
<td>4.91</td>
<td>71.6</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble</td>
<td>2.93</td>
<td>45.8</td>
</tr>
<tr>
<td>Expt. III</td>
<td>Acid-soluble</td>
<td>2.23</td>
<td>47.9</td>
</tr>
<tr>
<td></td>
<td>Acid-insoluble</td>
<td>1.97</td>
<td>52.7</td>
</tr>
</tbody>
</table>

* Two series of cultures were grown in the presence of 1.5 mM theophylline or 1.5 mM theophylline plus 1.5 mM dbcAMP. After 18 hr growth each culture flask received 10 µl of a \(^3H\)-theophylline solution (final activity: 2.5 μCi/ml of medium) and incubation was continued for an additional 6 hr. Each value represents the average radioactivity in either the TCA-soluble or TCA-insoluble fractions normalized to protein content in the insoluble fraction from 5 cultures.

Incorporation of \(^3H\)-Theophylline into Acid-Soluble and Acid-Insoluble Cell Fractions

Substantial quantities of tritium label from \(^3H\)-theophylline were incorporated into cells incubated in the presence of this

### Figure 2. Time course of \(^3H\)-theophylline incorporation into TCA-soluble and TCA-insoluble cell fractions. Cultures grown in the presence of 1.5 mM \(^3H\)-theophylline were chilled on ice and harvested at 4°C at the indicated times: (□—□) acid-soluble incorporation, (●—●) acid-insoluble incorporation. Each point represents the average of 4 replicate cultures.

### Figure 3. Incorporation of \(^3H\)-theophylline into TCA-soluble and TCA-insoluble cell fractions as a function of theophylline concentration. Cultures exposed to the indicated concentrations of \(^3H\)-theophylline were grown for 6 hr and then chilled on ice and harvested at 4°C: (□—□) acid-soluble incorporation; (●—●) acid-insoluble incorporation. Each point represents the average of 4 cultures.
agent for 6 hr (Fig 2 and 3). Label was rapidly incorporated into the acid-soluble cell fractions during the first 30 min after changes to H-theophylline-containing medium; this incorporation continued to increase between the first and sixth hr but at a much lower rate. The rate of incorporation of label into acid insoluble fractions increased steadily during the first 2 hr and then continued at a constant rate thereafter. In another experiment in which cells were exposed for 6 hr to various concentrations of H-theophylline the amount of label found in the acid-insoluble fractions increased with higher theophylline concentrations up to, but not above, 5 mM whereas the amount of label in the acid-soluble fractions increased with higher theophylline concentration over the entire range of concentrations tested (Fig 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>dpm/mg protein × 10−5</th>
<th>% Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. I</td>
<td>Expt. II</td>
</tr>
<tr>
<td>DNase</td>
<td>1.72</td>
<td>0.56</td>
</tr>
<tr>
<td>RNase</td>
<td>1.95</td>
<td>1.35</td>
</tr>
<tr>
<td>None</td>
<td>3.46</td>
<td>2.20</td>
</tr>
</tbody>
</table>

Harvested cells from 10–12 cultures previously grown in the presence of H-theophylline (18.4 Ci/m mole) were precipitated with cold 10% TCA and the insoluble fractions were digested with DNase or RNase and residues analyzed as described in the text.

**Nuclease Digestion of H-Theophylline-Labeled TCA-Insoluble Fractions**

Because theophylline is closely related to xanthine, a naturally occurring precursor of purine bases, we investigated the possibility that theophylline might also serve as a precursor to nucleic acids; we have observed that a large percentage of the incorporation of the acid-insoluble (but not the soluble) label could be inhibited by cordycepin. When TCA-extracted cell material which had been previously labeled over a 24 hr period with H-theophylline was suspended in buffer containing RNase or DNase, nuclease digestion was complete within 30–60 min. In these experiments 43–44% of the incorporated label was hydrolyzed by RNase while 50–74% of this label was hydrolyzed by DNase (Table IV). 14C-nucleosides incorporated into the same cultures over a 4 hr period exhibited essentially the same time course of hydrolysis by RNase and DNase; 62% and 36% of the 14C label were released as acid-soluble material by DNase and RNase respectively.

**Thin-Layer Chromatography of Nuclease Digests of Cells Labeled with H-Theophylline and 14C-Nucleosides**

Nucleotides resulting from RNase and DNase digests of cells labeled with H-theophylline and 14C-nucleosides were separated on PEI cellulose thin-layer sheets (Fig 4 and 5). Under the conditions used the Rf values for AMP, CMP, GMP and UMP at pH 3.5 were 0.53, 0.66, 0.34 and 0.58 respectively and 0.36, 0.53, 0.35 and 0.45 respectively at pH 4.4. dAMP, dCMP,
dGMP and TMP chromatographed with R\textsubscript{f} values of 0.44, 0.51, 0.39 and 0.48 respectively at pH 3.5 and 0.54, 0.59, 0.42 and 0.66 respectively at pH 4.4.

The distribution of \textsuperscript{3}H and \textsuperscript{14}C label in RNase digest was closely associated with AMP, CMP, and UMP (Fig 4; data not shown for GMP). However, the upper panels in Fig 4 also show the existence of a broad peak occurring midway between UMP and CMP (column G) in rows 11-13 along the first dimension and which increased in magnitude from row 12 to row 13 as the narrower \textsuperscript{14}C peak corresponding to CMP in the same panels simultaneously decreased. In contrast, the pattern of \textsuperscript{3}H label found over the AMP, GMP, and UMP markers exactly matched the distribution of \textsuperscript{14}C label in these regions.

Similarly, the distribution of \textsuperscript{3}H label in DNase digests is almost identical to that shown for the \textsuperscript{14}C label corresponding to the normal deoxyribonucleotides (Fig 5). However, 2 regions exist where the \textsuperscript{3}H and \textsuperscript{14}C labels overlap but do not coincide. The \textsuperscript{3}H peaks associated with dGMP (columns A-D) in rows 6 and 12 were found in fractions displaced from the \textsuperscript{14}C peak by 0.5 cm.

**Melanogenesis During Loss of Incorporated \textsuperscript{3}H-Theophylline**

Melanotic activity of cells pretreated with \textsuperscript{3}H-theophylline for 22 hr continues to be induced over a 50 hr period after the resuspension of cells in fresh medium lacking theophylline (Fig 6A). The stimulation of melanogenesis was greatest between 24-50 hr after the introduction of theophylline-free medium—a time during which the acid-soluble pool of \textsuperscript{3}H label drops from about 20% to 4% of its initial value with a half life of about 11 hr (Fig 6B). Significantly, TCA-insoluble \textsuperscript{3}H label remained extremely stable over the 50 hr of the experiment; the decrease in values in Fig 6B was largely the result of an increasing net protein synthesis over time rather than loss of \textsuperscript{3}H label from insoluble fractions.

**DISCUSSION**

In a previous paper [11], we observed that theophylline was a potent stimulator of melanin biosynthesis in RPMI 3460 cells and we suggested that this stimulation was, at least in part, unrelated to an increase in intracellular cAMP levels, the commonly assumed mechanism for theophylline action. The possibility that other phosphodiesterase inhibitors exert effects not mediated by cAMP has recently been suggested by others [17]. It is unlikely that the small initial elevation of cAMP levels could account for the subsequent melanotic stimulation which continues to increase for as many as 2-3 cell doublings after the initial rise in cAMP levels and during a time when cAMP levels were separately depressed. The data in Table I essentially confirm this supposition. Removal of theophylline after 12 hr should make little difference in the final level of melanotic activity attained in a 36 hr period if the determining factor had been an early stimulation of cAMP levels. Since this was not the case it can be assumed that theophylline exerts a critical effect on melanogenesis during the 12-36 hr period of treatment, a time during which the presence of the agent causes a reduction in cAMP levels. Prolonged exposure to theophylline might lead to an increase in phosphodiesterase activity as has been reported in a study of the related compound, methyl isobutyl xanthine [18]. The inability of theophylline to stimulate cAMP levels in some cell types has been noted by others [19].

Our findings that \textsuperscript{3}H label was rapidly incorporated into cells exposed to \textsuperscript{3}H-theophylline provides the basis for an alternative view of theophylline action. The apparently paradoxical inhibitory effect of dbcAMP on theophylline-induced melanogenesis can be explained by the finding that the uptake and incorporation of \textsuperscript{3}H-theophylline into cells is also reduced by exposure to dbcAMP. These findings suggest: (1) that the induction of melanogenesis by theophylline is related to the uptake and metabolism of the agent, and (2) that this effect of theophylline might emanate from either of 2 intracellular pools—one of which contains a form of the effector incorporated into macromolecules. The acid-soluble \textsuperscript{3}H pool is probably too labile (see Fig 6) to account for the stimulation of melanogenesis occurring 50 hr after the removal of exogenous theophylline. In contrast, the \textsuperscript{3}H label in the acid-insoluble fraction of \textsuperscript{3}H-theophylline-labeled cells is quite stable. Theophylline-treated mouse melanoma cells exhibit enhanced melanotic activity for as long as 5 days after the change from theophylline-containing the theophylline-free culture medium [16]. These data are consistent
with the hypothesis that the melatonin effects of theophylline are not directly caused by acid-soluble theophylline and/or its metabolites.

Virtually all of the acid-insoluble ^3H label became soluble again when treated with RNase and DNase. Some of the ^3H label in both RNase and DNase digests chromatographed as what might be a new species on ion exchange thin-layer sheets although the majority of the ^3H-theophylline derivatives were indistinguishable from normal nucleotides by this procedure. The possibility that some nucleotide species may have been preferentially eluted when thin-layer sheets were washed with methanol precludes obtaining an estimate of the relative distribution of ^3H label among the normal nucleotide species. However, when ribonucleosides from ^3H-theophylline-treated cells were resolved on a Dowex column, 80% of the ^3H label was associated with purine nucleotides, a result consistent with the purine ring structure of theophylline. The direct conversion of theophylline into purine nucleotides could occur through N-demethylation of the effector to yield xanthine, a substrate for xanthine phosphoribosyl transferase whose product is xanthinic acid, in turn, the direct precursor of both adenylc and guanylic acids. Goth and Cleaver [21] have reported that caffeine can serve as a precursor to purines in a variety of cultured cell types. These investigators also proposed a model of methyl xanthine metabolism based on demethylation resulting in the formation of xanthine. Although the unidentified activities in Fig 4 and 5 are too small to assess their significance, it is likely that they are related in some way to the role of theophylline as a nucleic acid precursor. A close parallel to the situation described here has been reported by Miller, Lawley and Shah [20] in which the incorporated label from O^-methylglycine into RNA and DNA was found in guanosine, thymidine, deoxyguanosine and an unidentified product.

Purine analogs are known to modify nucleic acid structure and function as a result of their ability to serve as nucleic acid precursors [10]. Recently, it has been shown that methylated purines in particular play an important role in the processing and usage of RNA's [22–24]. Unfortunately, it remains difficult at present to suggest an obvious mechanism by which the methylated purine, theophylline, might enhance melanin bio-synthesis. The important point is that this drug, commonly assumed to act via the cAMP-phosphodiesterase pathway, can enter into and/or possibly alter normal nucleic acid metabolism. An interesting comparison is that hypoxathine, in sufficiently high concentrations, can act as an inducer of differentiation in erythroid cells transformed by Friend leukemia virus [25].

After UV irradiation, DNA repair synthesis is inhibited by theophylline and caffeine [26] but not by cAMP [27] although the possibility that methylxanthine incorporation into DNA resulting in impaired excision and/or ligase activity has not been investigated. Studies of other systems may reveal new effects of theophylline which are not easily explained by effects on the cAMP-phosphodiesterase pathway. An alternative theory that the primary site of theophylline action is on nucleic acid metabolism could explain many diverse phenomena associated with theophylline treatment.

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REFERENCES


