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EFFECTS OF THE CHIRAL ISOMERS OF *ERYTHRO*- AND *THREO*-9-(2-HYDROXY-3-NONYL)ADENINE ON PURINE METABOLISM IN SARCOMA 180 CELLS

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Abstract—The effects of the chiral isomers of *erythro*- and *threo*-9-(2-hydroxy-3-nonyl)adenines (EHNA and THNA) on purine metabolism in Sarcoma 180 cells have been determined. At concentrations of 10–80 μM [10- to 1000-fold greater than their K_i values with adenosine deaminase (ADA)], all isomers inhibited purine salvage and biosynthesis *de novo*. Although (+)-EHNA, the most potent ADA inhibitor, exerted the greatest effects, there was no direct correlation between the potency of ADA inhibition and the secondary effects on purine metabolism, e.g. (+)-EHNA is about 2-fold more inhibitory than (−)-EHNA in blocking purine base incorporation but about 250-fold more potent as an inhibitor of ADA (K_i of (+)-EHNA = 2 nM; K_i of (−)-EHNA = 500 nM [Bessodes *et al.*, *Biochem. Pharmac.* **31**, 879 (1982)]). All the isomers inhibited the incorporation of radiolabeled purine bases (adenine, guanine and hypoxanthine) and nucleosides (guanosine and inosine) into acid-soluble nucleotides and of glycine into 5′-phosphoribosyl-formylglycineamide. Unlike the results of Henderson *et al.* [*Biochem. Pharmac.* **26**, 1967 (1977)] with Ehrlich ascites cells, the incorporation of adenosine into nucleotides was only slightly inhibited in Sarcoma 180 cells. (+)-EHNA did not inhibit the activities of 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase, purine phosphoribosyltransferases or nucleotide kinases in cell extracts. Accumulation of PRPP was inhibited only under conditions that fostered rapid synthesis.

Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA†), a semi-tight-binding inhibitor of adenosine deaminase (ADA) [1, 2], potentiates the anti-cancer and anti-viral activities of adenosine analogs that are substrates of ADA [3, 4]. To date 2′-deoxycoformycin, rather than EHNA, has been selected for clinical trials, a decision that appears to be based on two considerations: (1) the rationale that a tight-binding ADA inhibitor such as dCF would protect adenosine analogs more effectively from deamination, and (2) the observation that EHNA affects several aspects of cellular purine metabolism [5] and may therefore be a less specific drug than dCF.

Some of the toxic effects seen in clinical trials with dCF, i.e. profound lymphocytopenia, hemolytic anemia and CNS disturbances [6–9], may result from the prolonged inhibition of ADA in many tissues which permits accumulation of dATP to the extent that it replaces ATP in patient erythrocytes [7, 8]. Elevation of dATP has been found in mice treated four times daily with 50 mg/kg EHNA for 54 hr but not when the drug was administered in single daily doses of 50 mg/kg for 7 days [10]. Thus, it may be advantageous to use a semi-tight-binding inhibitor such as EHNA for short-term, controlled inhibition of ADA, particularly in circumstances where immunosuppression is undesirable.

An important question is whether the effects of EHNA on purine metabolism might cause unwanted toxicities or interfere with the potentiation of adenosine analogs. Henderson *et al.* [5] reported that 76 μM EHNA, preincubated for 20 min with Ehrlich ascites cells, inhibited the incorporation of purine bases and adenosine into nucleotide pools and of glycine into FGAR. The greatest inhibition of incorporation occurred with adenosine (about 75% with 76 μM EHNA and 40% with the lowest dose tested, 3.6 μM EHNA) but was reversed if the cells were washed twice before adding adenosine. In comparison, dCF had little effect on the incorporation of

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† Abbreviations: EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; THNA, *threo*-9-(2-hydroxy-3-nonyl)-adenine; (+)-EHNA, (+)-*erythro*-9-(2*S*-hydroxy-3*R*-nonyl)adenine; (−)-EHNA, (−)-*erythro*-9-(2*R*-hydroxy-3*S*-nonyl)adenine; (±)-EHNA, racemic mixture of (+)-EHNA and (−)-EHNA; (+)-THNA, (+)-*threo*-9-(2*R*-hydroxy-3*R*-nonyl)adenine; (−)-THNA, (−)-*threo*-9-(2*S*-hydroxy-3*S*-nonyl)adenine; ADA, adenosine deaminase; dCF, 2′-deoxycoformycin, covidarabine, pentostatin, or [3-(2′-deoxy-β-D-erythropentofuranosyl)-6,7,8-trihydroimidazo[4,5-*d*][1,3]diazepin-8-(R)-ol]; FGAR, 5′-phosphoribosyl-formylglycineamide; PRPP, 5-phosphoribosyl-1-pyrophosphate; APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; NDP kinase, nucleoside diphosphokinase; and Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid.

purine bases and glycine and inhibited adenosine incorporation by about 50%. The latter effect was also reversible by washing and the authors attributed the inhibition by both EHNA and dCF to a blockage of adenosine transport into the cell. Inhibition of adenosine metabolism by EHNA and dCF has also been observed in rat polymorphonuclear leucocytes [11]. The incorporation of adenosine into nucleotides was inhibited by 60 and 80% by 2 and 20 μM EHNA respectively.

The EHNA employed in the studies cited above was a racemic mixture, and it was not known whether one or both isomers were responsible for the ADA inhibition and the effects on purine metabolism. Recently, the chiral isomers of EHNA and of THNA were synthesized by an unequivocal route [12] and tested as inhibitors of ADA [13]. It was determined that (+)-EHNA is the most potent inhibitor of human erythrocytic ADA (K_i = 2 nM), whereas (−)-EHNA is relatively weak (K_i = 500 nM). The (+) and (−) isomers of THNA have intermediate affinities for ADA (K_i = 122 and 80 nM). The present studies were undertaken to determine the effects of the individual isomers of EHNA and THNA on purine metabolism in Sarcoma 180 cells.

MATERIALS AND METHODS

Materials. The chiral isomers of EHNA and THNA were synthesized by procedures reported elsewhere [12]. Racemic EHNA was provided by Dr. John Douros of the National Cancer Institute, Bethesda, MD. The ^{14}C-labeled chemicals were obtained from the New England Nuclear Corp., Boston, MA. Purification of the [^{14}C]glycine [14] was not required because less than 0.18% of the radioactivity applied to an AG 1-X8 formate column was retained after elution with 0.5 M formic acid. [8-^{14}C]Inosine was synthesized enzymatically from [8-^{14}C]adenosine with calf intestinal ADA. The conversion of adenosine to inosine was 97% as determined by cellulose thin-layer chromatography. Other special reagents were: ATP, GTP, GDP, GMP, adenosine, glycine, glutamine, 5-phosphoribosyl-1-pyrophosphate (PRPP), phosphoenolpyruvate, type II rabbit muscle lactate dehydrogenase and type II rabbit muscle pyruvate kinase (Sigma Chemical Co., St. Louis, MO); ADP, AMP, IMP and guanine (P-L Biochemicals, Milwaukee, WI); azaserine (Calbiochem-Behring Corp., La Jolla, CA); and Fischer's medium without sodium bicarbonate (Grand Island Biological Co., Grand Island, NY). Partially purified human erythrocytic HGPRT was prepared by the New England Enzyme Center (Boston, MA) by a large-scale method [15]. Sarcoma 180 cells were grown in CD_1 mice. Cells were harvested 6–7 days after intraperitoneal injection of 5–6 × 10^6 cells.

Incorporation of purine bases and nucleosides into nucleotide pools. Sarcoma 180 cells were harvested at room temperature in Fischer's medium containing 25 mM phosphate, pH 7.4, and washed three times by centrifugation for 30 sec at 600 *g*. A stock suspension of 4% (v/v) cells was prepared in the same medium.

The incorporation of the radiolabeled substrates into nucleotide pools was carried out essentially as described by Henderson *et al.* [5]. [8-^{14}C]Adenine (sp. act. 55.6 mCi/mmole), [8-^{14}C]guanine (sp. act. 54.67 mCi/mmole), [8-^{14}C]hypoxanthine (sp. act. 58 mCi/mmole), [8-^{14}C]guanosine (sp. act. 49.2 mCi/mmole) and [8-^{14}C]inosine (sp. act. 45.5 mCi/mmole) were used without dilution of the radioactivity; [8-^{14}C]adenosine (sp. act. 45.5 mCi/mmole) was diluted with unlabeled adenosine to give a specific activity of 22.8 mCi/mmole in the final reaction mixture. Details of the incubation and extraction procedures are given in Fig. 1. Chromatography of the reaction products was carried out on PEI-cellulose (Brinkmann Instruments, Westbury, NY) as described elsewhere [16] except that the thin-layer plates were prewashed only with methanol–water (1 : 1) overnight and air-dried before use. Total radioactive acid-soluble nucleotides were cut from the origin after methanol–water elution to provide the data in Figs. 1 and 2; subsequent stepwise elution with formic acid was used to separate the nucleotides for the data in Figs. 3 and 4. Radioactivity was counted in 8 ml of Betafluor (National Diagnostics, Sommerville, NJ).

Incorporation of glycine into 5′-phosphoribosylformylglycineamide (FGAR). Sarcoma 180 cells were harvested, washed and suspended at a concentration of 5% (v/v) in calcium-free Krebs–Ringer medium [17]. The rate of purine biosynthesis *de novo* was determined as described by Crabtree *et al.* [14] with slight modifications. Cell incubations with 0.105 μCi of [2-^{14}C]glycine/600 μl reaction volume were carried out as reported by Henderson *et al.* [5]. Details of the incubation and extraction procedure are given in Fig. 5. Extracts were chromatographed on AG 1-X8 resin (Bio-Rad Laboratories, Richmond, CA). Radioactivity was counted in 6 ml of ACS fluid (Amersham, Arlington Heights, IL).

Determination of PRPP levels. Sarcoma 180 cells were collected in Puck's saline G and washed three times by centrifugation at 600 *g* for 30 sec. Cellular PRPP stores were depleted* by incubating 5% cell suspensions for 10 min at room temperature in saline buffered with 1 mM Hepes, pH 7.4. The cells were centrifuged and resuspended at 5% (v/v) in 1 ml of Fischer's medium containing 25 mM phosphate, pH 7.4, or in 1 ml of high phosphate medium (50 mM phosphate, pH 7.4; 75 mM NaCl; 10 mM glucose; 2 mM $MgSO_4$). Incubations were carried out in the presence and absence of 80 μM EHNA for 1 hr at 37° on a shaking water bath under an air atmosphere. The cells were centrifuged, resuspended in 0.3 ml of ice-cold 1 mM potassium phosphate, pH 7.4, containing 1 mM EDTA, boiled for 65 sec, and cooled rapidly on an ice bath. The boiled samples were centrifuged for 5 min at 2000 *g*, and 50 μl aliquots of the supernatant fractions were assayed for PRPP by the method of Green and Martin [18].

Enzyme assays. Cell extracts for assaying kinase and phosphoribosyltransferase activities were prepared by washing the Sarcoma 180 cells with saline and lysing 30% (v/v) suspensions of the cells in 100 mM Tris–HCl, pH 7.4, by sonication. Enzyme

* F. Burgess, unpublished observation.

activities were determined in the supernatant fluid after centrifugation for 1 hr at 100,000 *g*. HGPRT and APRT activities were measured by trapping radiolabeled GMP and AMP on DE81 filter discs (Whatman Inc., Clifton, NJ) by a modification of the method of Parks *et al.* [19]. The reaction mixtures contained 4.5 to 50 μM ^{14}C-labeled purine base, and 15 mM NaF. At various time intervals, 20 μl aliquots were placed on the filter discs which were then washed repeatedly with 1 mM ammonium formate by suction. Radioactivity on the discs was counted in ACS fluid after extraction with 1 ml of 1 N HCl. AMP kinase, GMP kinase and NDP kinase were measured spectrophotometrically at 340 nm by a coupled assay [15].

Extracts for PRPP synthetase were prepared by the procedure of Yip *et al.* [20], and enzyme activity was measured as described [21] except that the reaction was coupled to HGPRT instead of APRT. Each reaction mixture contained cell extract equivalent to 2.9 μl of packed cells, 200 μM ribose-5-phosphate, 105 μM guanine (0.5 μCi of [^{14}C]guanine) and 0.02 units of partially purified human erythrocytic HGPRT.

RESULTS

Effects on purine salvage pathways. The incorporation of adenine, guanine and hypoxanthine into the nucleotide pools of Sarcoma 180 cells was inhibited by (±)-EHNA as well as by all the EHNA and THNA isomers. Figure 1 shows that inhibition was greatest with guanine and hypoxanthine and less with adenine. The (+)-EHNA isomer, which is the most potent ADA inhibitor, also had the most profound inhibitory effect. The (−)-EHNA isomer was significantly less inhibitory, especially at higher concentrations, and gave results that were similar to racemic (±)-EHNA. The THNA isomers generally gave less inhibition than the EHNA isomers and exerted their greatest activity against guanine incorporation. The order of inhibitory potency was not constant with the THNA isomers: guanine incorporation was inhibited more strongly by (−)-THNA and hypoxanthine more by (+)-THNA. When the effect of 80 μM (+)-EHNA was tested on the HGPRT and APRT activities in extracts of Sarcoma 180 cells, no inhibition was observed even at low concentrations (4.5 μM) of the adenine and guanine substrates.

Whereas guanine, hypoxanthine and adenine incorporations were inhibited 68, 67 and 55%, respectively, by 80 μM (+)-EHNA, adenosine incorporation into acid-soluble nucleotides was only slightly affected, as shown in Fig. 2. Since these results suggested that EHNA might block base transport, the incorporation of ^{14}C-labeled inosine and guanosine was examined. The nucleosides would presumably enter the cell via a nucleoside transport mechanism and be cleaved by purine nucleoside phosphorylase (which is not inhibited by EHNA) to release the radiolabeled bases. Figure 2 shows that incorporation of inosine and guanosine was also strongly inhibited by 10–80 μM (+)-EHNA. The other isomers were not tested with inosine or guanosine and showed slight or no inhibition of adenosine uptake (results not shown).

The ratios of adenine nucleotides to guanine nucleotides formed from [^{14}C]hypoxanthine were determined after chromatographic separation of the acid-soluble nucleotides. Figure 3 shows that the A/G ratio of nucleotides formed from hypoxanthine dropped from *ca.* 11 in the control to 5.6 and 6.3 with 80 μM (+)-EHNA and (±)-EHNA respectively. Incorporation of radiolabeled substrate into adenine nucleotides decreased by 60–65%, whereas that into guanine nucleotides decreased by 28–30%. Although the A/G ratio decreased as the concentra-

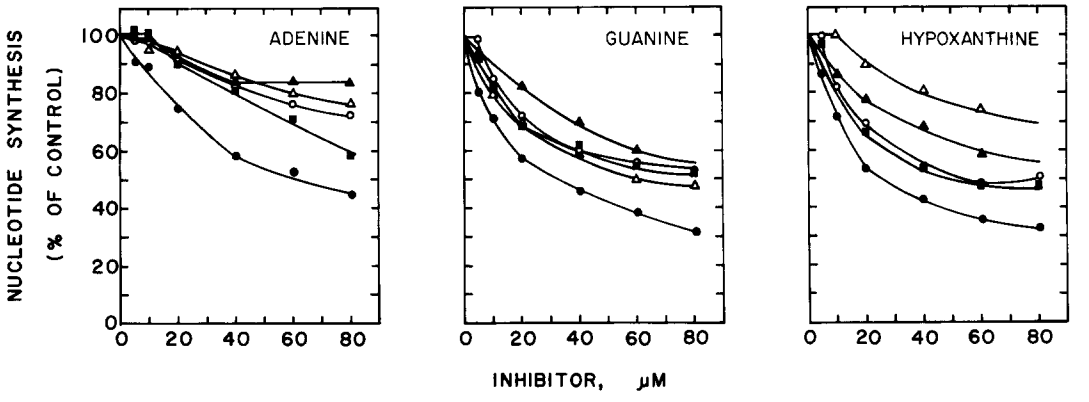

Fig. 1. Effects of the EHNA and THNA isomers on nucleotide synthesis from purine bases. Cells were preincubated for 20 min with various concentrations of (±)-EHNA (■), (+)-EHNA (●), (−)-EHNA (○), (+)-THNA (▲) and (−)-THNA (△). After addition of ^{14}C-labeled adenine, guanine or hypoxanthine, the incubation was continued for 30 min at 37° in an air atmosphere. The 100 μl reaction volumes contained 50 μl of cells, 25 μl of inhibitors and 25 μl of substrate, all in modified Fischer's medium, to give final concentrations of 2% cells, 100 μM substrate and 0–80 μM inhibitor. The reactions were stopped by freezing in a dry-ice/ethanol bath. The protein was precipitated by adding 10 μl of 2.24 N perchloric acid before thawing the solutions at 4°. The extracts were neutralized with 10 μl of 2.24 N KOH and centrifuged at 4°. Aliquots of 10 μl were chromatographed and total radioactivity in acid-soluble nucleotides was determined and expressed as a percentage of the radioactivity incorporated in the absence of inhibitor. The results are averages of duplicate determinations and were essentially the same in two separate experiments.

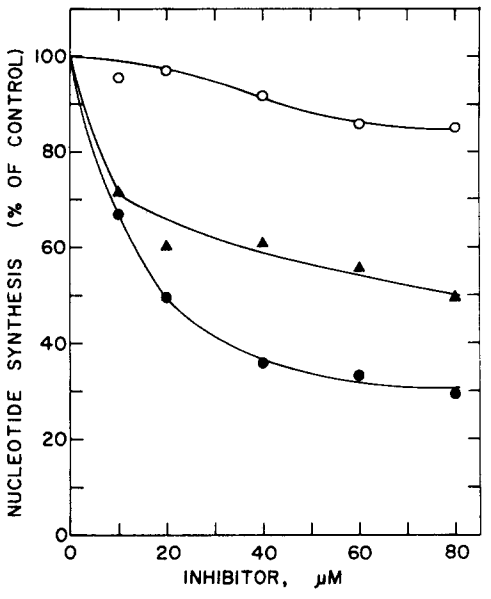

Fig. 2. Effects of (+)-EHNA on nucleotide synthesis from purine nucleosides. The procedures used are described in Fig. 1 except that the reaction mixtures contained 200 μM [8-^{14}C]adenosine (○), 100 μM [8-^{14}C]guanosine (▲) or 100 μM [8-^{14}C]inosine (●).

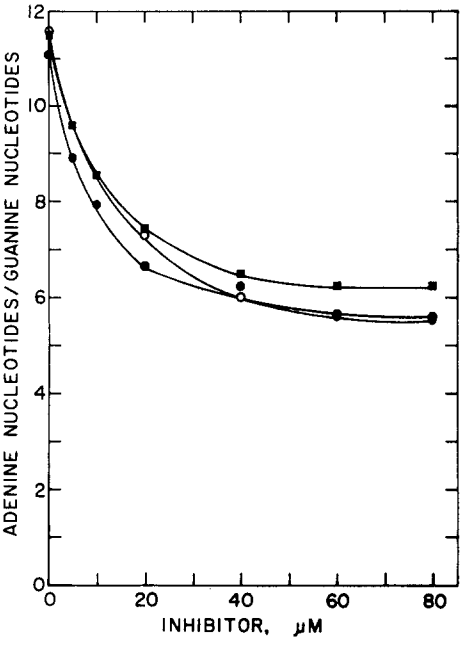

Fig. 3. Effects of EHNA on the conversion of hypoxanthine and inosine to adenine nucleotides and guanine nucleotides. Cells were incubated in the presence of (±)-EHNA (■) or (+)-EHNA (●) with [8-^{14}C]hypoxanthine as substrate, and (+)-EHNA with [8-^{14}C]inosine (○) as substrate, as described in Fig. 1. Radioactivity was determined in IMP, AMP, ADP, ATP, GMP, GDP and GTP. The values given are averages of duplicate determinations. Similar results were obtained in two separate experiments.

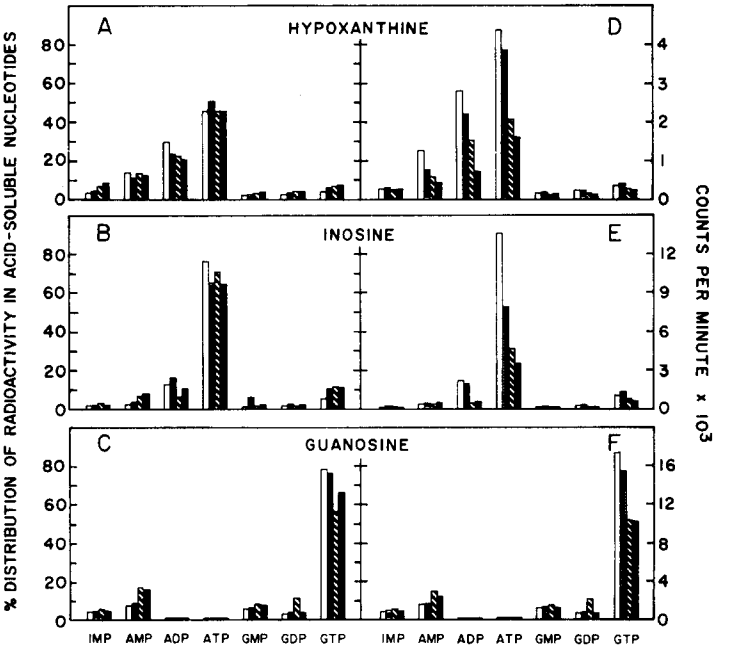

Fig. 4. Effects of (+)-EHNA on the distribution of radioactivity in acid-soluble nucleotides synthesized from hypoxanthine, inosine and guanosine. Cells were incubated in the presence of various concentrations [0 μM (open bar), 20 μM (horizontal hatch), 40 μM (diagonal hatch) and 80 μM (closed bar)] of (+)-EHNA, and with hypoxanthine, inosine or guanosine as substrates as described in Figs. 1 and 2. Radioactivity in IMP, AMP, ADP, ATP, GMP, GDP and GTP was determined as described in Fig. 3. In panels A, B and C, the distribution of radioactivity was calculated by dividing the counts per min for each nucleotide by the counts per min in total acid-soluble nucleotides at a given concentration of (+)-EHNA. Panels D, E and F represent the actual radioactivity (counts per min) recovered in each nucleotide species.

tion of EHNA increased, the relative distribution of radioactivity among the mono-, di- and triphosphate nucleosides was almost unaffected by the presence of (±)-EHNA or (+)-EHNA (Fig. 4). Similar results were obtained with inosine and (+)-EHNA (Figs. 3 and 4) except that, consistently, a higher percentage of the radioactivity in adenine nucleotides was in the form of ATP. Whereas the sharp drop in the A/G ratio of nucleotides derived from hypoxanthine and inosine suggests that EHNA blocks the IMP → AMP pathway, the distribution pattern of radioactivity from guanosine (Fig. 4) shows a slight increase in AMP with increasing EHNA concentrations. Interestingly, although AMP represented 7.6 to 16.5% of the radioactive nucleotides formed from guanosine, ATP + ADP accounted for less than 2%. The synthesis of AMP from guanosine in Sarcoma 180 cells significantly exceeds the synthesis of AMP from guanine reported for Ehrlich ascites cells [16] or mixed human leucocytes [17] and the radioactive ATP/AMP ratio is reversed. Assays of the nucleotide interconverting enzymes, AMP kinase, GMP kinase and NDP kinase, in Sarcoma 180 cell extracts gave the same results in the presence or absence of 80 μM (+)-EHNA.

Effects on purine de novo *biosynthesis.* All the EHNA and THNA isomers profoundly reduced the utilization of glycine for the synthesis of purines. The formation of radiolabeld FGAR was inhibited by more than 50% at 10 μM concentrations of all the isomers [except (−)-THNA] and 60–70% as the inhibitor concentrations were increased from 20 to 80 μM (Fig. 5).

Since purine biosynthesis *de novo* and the salvage of purine bases all require PRPP as a co-substrate, the effects of EHNA on PRPP synthetase in Sarcoma 180 cell extracts and on PRPP levels in intact cells were studied. The PRPP synthetase activity of Sarcoma 180 cell extracts was not inhibited by 80 μM (+)-EHNA. Intact cells that were depleted of PRPP by incubation in glucose-deficient medium attained the same low PRPP levels (61.7 ± 5.1 and 59.3 ± 3.8 nmoles/10^9 cells) after incubation in the modified Fischer's medium with or without 80 μM (±)-EHNA respectively. However, when depleted cells were incubated in medium containing 50 mM phosphate and 10 mM glucose, 19-fold higher PRPP levels were attained and 80 μM (±)-EHNA reduced these by 35% from 1160 ± 78 to 752 ± 34 nmoles/10^9 cells.

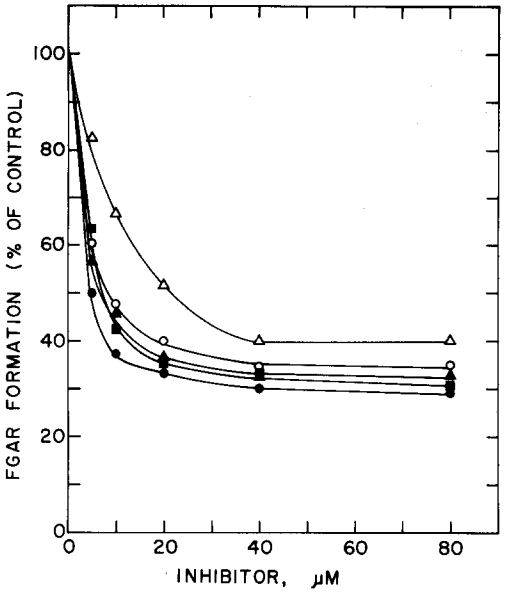

Fig. 5. Effects of EHNA and THNA isomers on purine biosynthesis *de novo*. Sarcoma 180 cells (500 μl of a 5% suspension) were incubated for 20 min at 37° in modified Krebs–Ringer medium contained azaserine (10 μM final concentration) and 0–80 μM concentrations of (±)-EHNA (■), (+)-EHNA (●), (−)-EHNA (○), (+)-THNA (▲) and (−)-THNA (△). After addition of 100 μl of 2 mM glutamine and 1 mM [^{14}C]glycine, the incubation was continued for 1 hr and stopped with 150 μl of 20% perchloric acid. The solutions were chilled and neutralized to pH 6.5 to 7.5 with 130 μl of 5 N KOH. After centrifugation, 0.7 ml of the supernatant fluid was applied to a 12 × 28 mm AG 1-X8 column and unreacted glycine was eluted with 30 ml of 0.5 M formic acid. FGAR was eluted with 15 ml of 4 M formic acid, lyophilized and redissolved in 1 ml of distilled water from which 0.8 ml was counted. Results are expressed as a percentage of the radioactivity in FGAR in the absence of inhibitor and are averages of duplicate determinations. Essentially the same results were obtained in two separate experiments.

DISCUSSION

All the EHNA and THNA isomers inhibited the incorporation of purine bases into acid-soluble nucleotide pools, blocked *de novo* purine biosynthesis at an early stage, but had little or no effect on adenosine incorporation. Nucleotide synthesis from inosine and guanosine was also depressed by (+)-EHNA. This pattern of inhibitions suggests (although it does not prove) that these compounds interfere with purine metabolism rather than with transport processes. Since (+)-EHNA did not affect APRT, HGPRT or nucleotide kinase activities in Sarcoma 180 cell extracts, all the observed inhibitions might be explained by decreased availability of PRPP for base salvage and the first reaction of *de novo* synthesis. Yet EHNA did not affect PRPP synthetase activity in cells that were allowed to rebuild their depleted stores in the same modified Fischer's medium that was employed for the incorporation studies. Those cells, however, accumulated only about 0.06 μmole PRPP per 10^9 cells per hr. When the cells were incubated in a high phosphate medium that fostered the synthesis of 1.16 μmoles PRPP per 10^9 cells per hr, EHNA caused 35% inhibition. When the 2% cell suspensions were challenged with 100 μM concentrations of adenine, hypoxanthine or guanine (see Fig. 1), total radiolabeled nucleotide concentrations of 1.0 to 1.65 μmoles per 10^9 cells, respectively, were achieved during a 30-min incubation. A reasonable hypothesis is that EHNA interferes with the extremely high rate of PRPP production that is required for the nucleotide synthesis.

The mechanism by which EHNA exerts this influence cannot be deduced from the available data but may be unrelated to ADA inhibition. Although these inhibitions were more pronounced with (+)-EHNA than with the other isomers, there was no clear correlation to ADA inhibition. For example, (+)-

EHNA (K_i = 2 nM) is about 250-fold more potent than (−)-EHNA (K_i = 500 nM) as an inhibitor of ADA, but, as seen in Fig. 1, is only about twice as potent as an inhibitor of purine base incorporation. Furthermore, as seen in Fig. 1, the two THNA isomers, which are about 5-fold more potent as ADA inhibitors, are less active than (−)-EHNA in inhibiting the uptake of hypoxanthine.

These studies confirm and extend many of the observations of Henderson *et al.* [5] who found that EHNA has a number of secondary effects on the purine metabolism of Ehrlich ascites cells that are not readily explained by inhibition of ADA. Although, qualitatively, most of our findings with Sarcoma 180 cells are in agreement with those of Henderson *et al.* [5], the uptake of adenosine by Sarcoma 180 cells is not inhibited by any of the isomers in contrast to results with Ehrlich cells. Also, we observed more potent inhibition of guanine uptake. The discrepancies are probably due to metabolic differences in the two mouse tumors.

A significant point for the potential therapeutic application of EHNA is that the K_i of the more active isomer as an inhibitor of ADA is about 1000-fold lower than the concentrations at which these secondary effects on purine metabolism are seen. Since the K_i value of (+)-EHNA is 2 nM, an appropriate therapeutic tissue level might be in the order of 10^{-7} to 10^{-6} M, i.e. greater than 100 times K_i, but well below the concentration where these secondary effects on purine metabolism are observed. Therefore, it appears likely that it will be possible to disregard these secondary phenomena in considering EHNA as a candidate therapeutic agent for the potentiation of adenosine analogs.

In studies of deoxycoformycin with animals and in clinical trials, inhibition of ADA in erythrocytes and organs such as the liver was exceedingly prolonged [22]. It has been postulated that certain of the toxic effects observed in clinical trials of dCF may have been due to the greatly prolonged inhibition of ADA. Present evidence indicates that inhibition of intraerythrocytic ADA by dCF is permanent and that recovery of activity is due to the introduction of new erythrocytes into the blood stream from the bone marrow. These prolonged inhibitions might be avoided by use of a semi-tight-binding inhibitor such as (+)-EHNA which has a $T_{\frac{1}{2}}$ for dissociation of the enzyme–inhibitor complex of less than 5 min in contrast to the $T_{\frac{1}{2}}$ of the dCF–ADA complex of 29 hr. A recent report indicates that the duration of inhibition of whole body ADA in mice could be varied from 3 to about 12 hr dependent upon the dose of EHNA administered [10]. Thus, it may be possible to modulate the inhibition of ADA to correspond temporally with the administration of the adenosine analogs. A potentially significant observation is the failure of any of the EHNA-like isomers studied by us to inhibit adenosine uptake in Sarcoma 180 cells. Also, in current studies with (±)-EHNA, we have not detected any inhibition of adenosine uptake in the human colon carcinoma line, DLD-1 clone A, and the human lung tumor, LX-1.* This is an important point because if EHNA is to find a clinical role it will probably be in preventing the inactivation of adenosine analogs caused by reaction with ADA.

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