Biochemical Pharmacology, Vol. 25, pp. 2059–2065. Pergamon Press, 1976. Printed in Great Britain.

ORGAN-SPECIFIC DIFFERENCES IN THE METABOLISM OF N^6-(Δ^2-ISOPENTENYL)-ADENOSINE*

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(*Received* 22 *February* 1975; *accepted* 19 *December* 1975)

Abstract—N^6-(Δ^2-isopentenyl-adenosine (IPAR) is a modified ribonucleoside with antitumor activity limited at least in part by severe hepatotoxic effects observed in rodents and patients. Four hr after i.v. administration of IPAR[8-^{14}C] to rats, the drug is phosphorylated to the 5′-mono, di-, and triphosphates, and incorporated into RNA but not into DNA and protein of liver and small intestine. In contrast, in spleen and thymus, the drug is phosphorylated to the 5′-monophosphate (5′-IPAMP) only with no detectable incorporation of the drug into DNA, RNA or protein. The initial phosphorylation of IPAR to 5′-IPAMP in liver at 30 min is accompanied by a 70 per cent reduction in the ATP pool and an accumulation of 2.0 μmoles IPAMP/g. In thymus, the drug reduces the size of pyrimidine ribonucleotide pools, resulting in a 2- to 5-fold increase in the specific activity of [^{14}C]pyrimidine ribonucleotides. These effects may contribute to the understanding of the biochemical basis for selective cytotoxicity of N^6-isopentenyl adenosine.

N^6-(Δ^2-isopentenyl)-adenosine (IPAR) is an analog of adenosine that has been identified as a component of both yeast and mammalian tRNA [1–3]. The agent has broad biological activity, inhibiting the growth of *Escherichia coli* [4] and Sarcoma 180 cells in culture as well as cells derived from human myelogenous leukemia but not those derived from human lymphocytic leukemia [5]. In rats, the drug caused severe hepatotoxicity, as well as antiproliferative effects in intestinal mucosa, lymphoid tissues, bone marrow and regenerating liver [6].

In cultured mammalian cells (e.g. Sarcoma 180, mammary carcinoma TA-3, leukemia L1210, and canine kidney [7, 8]), as well as in chicken liver homogenates [9], IPAR has been reported to be phosphorylated only to the 5′-monophosphate. This metabolite apparently accounts for most of the inhibitory effects in these cells [7, 10]. In contrast, as we reported recently, IPAR is further phosphorylated to the IPADP and IPATP by liver in treated rats with this agent [11]. Moreover, Zimmerman and Chu [12] have also identified IPATP as a metabolite of IPAR in human blood. Because phosphorylation of IPAR in numberous cell systems is limited to formation of the 5′-monophosphate nucleotide, further phosphorylation of the agent *in vivo* was unexpected. In view of this striking difference in phosphorylation, it seemed possible that the nucleotidal forms of IPAR might contribute to its selectivity in animals. In the present study, formation of IPAR nucleotides in liver, intestine, thymus and spleen of treated rats was investigated. As described below, the results indicate that organ-specific differences in nucleotide formation may result in severe depletion of cellular ADP and ATP and in selective incorporation of the drug into RNA.

*This investigation was supported in part by Core Program Grant CA 13038 and by Project Grant CA 05298 from the National Cancer Institute, USPHS, and an allocation to Y. M. Rustum from the Institutional General Research Support Grant RR-05648-07, USPHS.

MATERIALS AND METHODS

Materials

Silica gel-coated plates (20 × 20 cm) were obtained from Analtech, Inc. Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1, calf intestinal mucosa, 380 units/mg), snake venom phosphodiesterase (orthophosphoric diester phosphohydrolase, EC 3.1.4.1, 10 mg/ml), and ribonuclease (polyribonucleotide 2′-oligonucleotidetransferase, EC 2.7.7.16, bovine pancreas, 10 mg/ml) were obtained from the Worthington Corp. The sources of other chemicals and solvents have already been described [13, 14]. IPAR[8-^{14}C] (50 μCi/μmole) was prepared by Dr. M. Fleysher [15] of this department and stored at −70° in 95% ethanol for several months without any detectable breakdown. The labeled compound was diluted 10-fold with unlabeled IPAR prior to administration to rats. IPAMP was kindly provided by Dr. Divekar of this department. Unlabeled IPAR was obtained from the Drug Research Development Branch of the National Cancer Institute (NSC batch 3856 001). Solutions of the drug were prepared immediately before use in 40% dimethylsulfoxide. Uridine [2-^{14}C] (UR[2-^{14}C] (50.0 mCi/m-mole) was obtained from Schwartz/Mann. Female Charles River CD Spraque–Dawley rats were used when they were approximately 5 weeks old and weighed 110–150 g.

Methods

Preparation of acid-soluble and -insoluble fractions. Rats were lightly anesthetized with ether and injected with IPAR (200 mg/kg) or IPAR[8-^{14}C] (15 μCi) in

0.5 ml in the femoral vein. At an appropriate time after administration of the drug, animals were anesthetized again and tissue (liver, spleen or small intestine) was gently forced out through an abdominal incision. The tissue was severed and dropped into liquid nitrogen (the time required to carry out this operation was about 10 sec). Frozen tissue was weighed and homogenized with 4 ml of 6% perchloric acid (PCA/g) in a prechilled blender for 1 min at 4°. After centrifugation, the acid-soluble fractions were neutralized to pH 7.0 with 2 N KOH, and the precipitate was removed 30 min later. Tissue extracts were freeze-dried and taken up in a minimum volume of 3 mM ammonium formate buffer at pH 4.4 and chromatographed on a microgranular DEAE-cellulose type DE-52 column [12]. The acid-insoluble fractions were washed three times with cold 6% PCA (10 ml each), once with saturated ammonium acetate, and once with ethanol (95%)-ether (3:1). This fraction was freeze-dried and kept at −70° for subsequent analysis.

Isolation of nucleic acids and protein. DNA, RNA and protein were isolated from the acid-insoluble fractions by the modification of the procedure of Kirby [16] and Kuroki and Heidelberger [17]. Fractions were each suspended in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 1.0 ml of 1% sodium dodecyl sulfate/g of tissue, and extracted with an equal volume of phenol *m*-cresol 8-hydroxyquinoline-water (500:79:95:55, by weight) for 30 min with stirring at room temperature. DNA, RNA and protein were separated [17] and each fraction was dissolved in a minimum volume of 0.1 M sodium acetate buffer. Each fraction was then gel-filtered on a Bio-Gel A 0.5-m (200–400 mesh) column (33 × 2.5 cm). This column was initially equilibrated with the same buffer and calibrated with calf thymus DNA, calf liver RNA and bovine serum albumin. The absorbed materials were eluted from this column with 0.1 M Tris buffer and fractions were collected for radioactivity and A_{260} nm absorbance estimations. The radioactive fractions were pooled, freeze-dried and dissolved in 2.0 ml of 0.1 M Tris buffer. To this solution, 0.3 mg ribonuclease (6100 units/mg) was added and incubated for 2 hr at 37°. After the incubation, 0.5 mg of snake venom phosphodiesterase (500 units/mg) in Tris-HCl buffer, pH 8.6, and 0.5 mg (190 units) of alkaline phosphatase were added, and the incubation was continued for 16 hr at 37° to hydrolyze nucleotides to nucleosides. Samples were freeze-dried and taken up in a minimum volume of distilled water for chromatographic identification.

Nucleotide digestions. Nucleotides eluted from the DEAE-cellulose type DE-52 columns were pooled, freeze-dried and digested to the ribonucleoside level as follows: dried material (10 A_{260} units) was dissolved in 1 ml of 0.1 M Tris-HCl buffer, pH 10.0, containing 1 mM $MgCl_2$. Alkaline phosphatase (4 units) was added and the mixture incubated at 37° for 1.5 hr. Four more units of the enzyme was added and the incubation terminated 1.5 hr later by freezing in liquid nitrogen. Control samples of ATP and IPAMP were treated concurrently in an identical manner. Following thin-layer chromatography (TLC) on Silica gel [11] it was found that 80–90 per cent of the digested nucleotides chromatographed as nucleosides (when samples were digested with alkaline phosphatase). Digestions to the ribonucleoside monophosphate level were carried out as follows: dried pool fractions (10 A_{260} units)* were dissolved in 1 ml of 0.1 M Tris HCl, pH 8.6, containing 5 mM $MgCl_2$. To this solution, 0.5 mg phosphodiesterase was added and incubated at 37° for 16 hr.

Silica gel and paper chromatography. After enzymatic digestion of nucleotides and RNA fractions, the released labeled components were identified by Silica gel chromatography using doubly deionized water as developing solvent. Digested samples with and without appropriate markers were separated and the u.v. absorbent components were scraped, extracted with 0.5 ml of 0.1 M ammonium formate (pH 7.0), and centrifuged at 2000 *g* for 1 min. Extractable materials (80–90 per cent recoveries) were then further identified by their absorbance ratios and by radioactivity measurements. Labeled nucleosides and nucleotides present in the enzymatically digested samples were also analyzed by ascending chromatography on Whatman 3MM paper with *n*-butanol glacial acetic acid water (20:3:7) [18].

Determination of radioactivity and absorbance. Radioactivity in various fractions before and after enzymatic digestions (0.01 to 0.2 ml) was measured in 4 ml of scintillation solution using a Packard Tri-Carb liquid scintillation counter. The counting solution consists of 5 g of 2,5-diphenyloxazole (PPO), 0.5 g of 1,4-bis-2-(phenyloxasolyl) benzene (POPOP) and 150 ml of Bio-Solv solution in 1 liter toluene. Concentrations of u.v. absorbing materials in acid-insoluble extracts and in fractions eluted from the DEAE-cellulose, type DE-52, column were determined spectrophotometrically at 260 and 280 nm using a Beckman DU-2 or American Instrument DW-2 spectrophotometer.

RESULTS

Effects of IPAR on liver purine ribonucleotide pools

Typical chromatographic separations of liver acid-soluble fractions (pooled from three rats) on a DEAE-cellulose, type DE-52, column are shown in Fig. 1. Two other experiments (not shown) gave similar results. Fractions 3–20 contain purine and pyrimidine nucleosides. The results in Fig. 1a also show four major purine ribonucleotide components identified as IMP (+TMP), AMP, ADP and ATP. Molar ratios of ATP/ADP in control chromatograms were about 3.0, which is close to those reported in the literature [19, 20].

The chromatographic profile of the acid-soluble fraction (pooled from three rats) of liver of rats injected i.v. with IPAR (200 mg/kg) 30 min prior to the rapid removal of tissue is shown in Fig. 1b. To quantitate the effects of IPAR on the pool of purine nucleotides, fractions eluted from the column (Fig. 1) in the areas of IMP, ADP and ATP were collected and their respective concentrations were determined using an extinction coefficient of 15.0 cm^2 μmole for adenosine nucleotides and 7.4 for IMP (at 260 nm.

*One A_{260} unit of nucleosides, nucleotides, or nucleic acid is defined here as the concentration that would have an absorbance of 1.0 at 260 nm in a cuvet with a 1-cm light path.

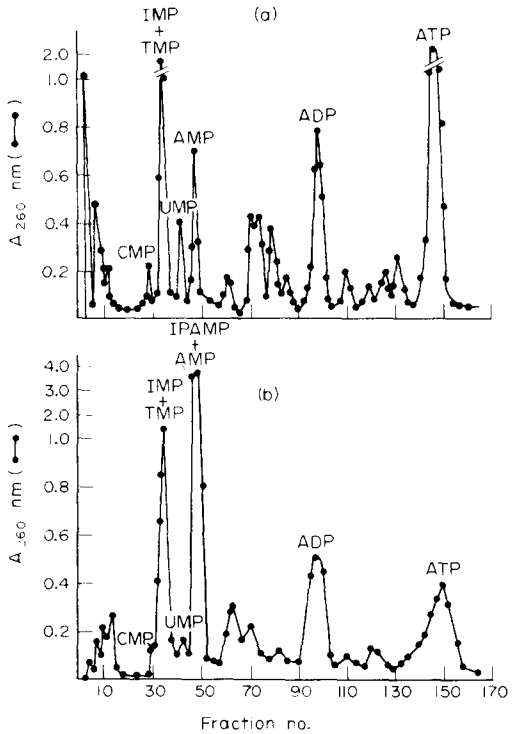

Fig. 1. DEAE-cellulose, type DE-52, column chromatographic separation of rat liver acid-soluble nucleotides. Extracts from 2 g of liver pooled from (a) three untreated or (b) three treated rats injected i.v. with IPAR (200 mg/kg) 30 min prior to sacrifice. Elution was with a linear gradient of 0.003 to 0.3 M ammonium formate, pH 4.4; 2.4-ml fractions were collected.

pH 7.0). The components eluted in the area of AMP were pooled, concentrated and chromatographed on Silica gel with water as developing solvent using authentic AMP and IPAMP as markers. The R_f values for AMP and IPAMP in this system were 0.87 and 0.50 respectively [13]. Components corresponding to the R_f of AMP and IPAMP were extracted from the gel and their concentrations were determined using an extinction coefficient of 20.0 $cm^2/\mu mole$ of IPAMP. Enzymatic digestion and Silica gel chromatography of the peak eluted in the ATP region of Fig. 1b revealed no detectable IPATP. The chromatogram, however, showed the presence of a component with an R_f that did not correspond to markers applied and which may have contributed to the broadening of the ATP peak in Fig. 1b.

The data summarized in Table 1 show that: (1) IPAR was phosphorylated only to the 5'-IPAMP and (2) the concentration of ATP was reduced by the drug to about 30 per cent of controls, and a significant decrease in the adenylate charge was also observed. Although the effects of IPAR on the pyrimidines were not quantitated in this study, a reduction in their nucleotide pool sizes seemed apparent in all experiments after IPAR administration.

Effects of IPAR on ribonucleotide pools in the thymus

Chromatographic separation of the ribonucleotides from the thymus of three rats treated i.v. with a single dose of IPAR (200 mg/kg) 30 min prior to administration of UR[2-^{14}C] is depicted in Fig. 2, which shows that UR [2-^{14}C] was phosphorylated into the corresponding uridine and also cytidine ribonucleotides. Identification of these components was made according to their elution positions on the calibrated DEAE-cellulose (type DE-52) column, by $A_{289/260}$ absorbance ratios, and by the other chromatographic systems described in Methods. Columns were calibrated, after they were first packed, by determining the elution positions of nucleosides and nucleotides in a standard mixture. The acid-soluble profile of the drug-treated sample is shown in Fig. 2b. Indications of the phosphorylation of IPAR to IPAMP was obtained from the high 280/260 nm absorbance ratios (0.65 instead of 0.15 for pure adenosine components) in the general area of AMP. The results also indicated that IPAR produced quantitative changes in the pyrimidine ribonucleotide pools in this tissue. To estimate the concentration of individual pyrimidine ribonucleotides in controls and drug-treated samples (Fig. 2a and 2b), fractions from two separate chromatograms were pooled, concentrated and analyzed, and the data are summarized in Table 2. The results indicate that IPAR produced a 2 to 5-fold increase in the specific activities of UMP, CDP, UDP, and CTP + UTP. IPAR also produced about a 30 per cent reduction in the ATP pool (from 1.2 μmoles/g of tissues to 0.80 μmole).

Table 1. Effects of IPAR (200 mg/kg) on purine ribonucleotide pools in rat liver

Components	Control* (μmoles/g)	IPAR-treated† (μmoles/g)	Ratios of IPAR-treated/control
IMP	1.05 ± 0.16	1.28 ± 0.15	1.22
AMP	0.18 ± 0.08	0.27 ± 0.07	1.50
IPAMP		2.06 ± 0.17	
ADP	0.72 ± 0.10	0.89 ± 0.13	1.24
ATP	2.35 ± 0.15	0.17 ± 0.13	0.30
Adenylate energy charge (AEC)‡	0.83 ± 0.02	0.29 ± 0.04	0.35

* Ribonucleotide components, separated on DEAE-cellulose, type DE-52, column, were hydrolyzed and identified by chromatography as the respective nucleosides (see Methods).

† IPAR was administered i.v. 30 min prior to removal of liver tissue. In each experiment, tissue from three rats was pooled and analyzed (average for three experiments ± S.D.).

‡ Adenylate energy charge calculated as:

$$\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP] + [IPAMP]}$$

Biotransformation of IPAR[8-^{14}C] *in rat tissues*

The data on chromatographic separation of acid-soluble nucleotides from liver, small intestine, spleen and thymus of rats treated i.v. with a single dose of IPAR [8-^{14}C] 4 hr prior to sacrifice are shown in Fig. 3. To minimize the breakdown of nucleoside triphosphates during tissue removal, only one organ was removed from each treated rat.

Figure 3a shows a chromatographic separation of liver acid-soluble nucleotides. Two radioactive

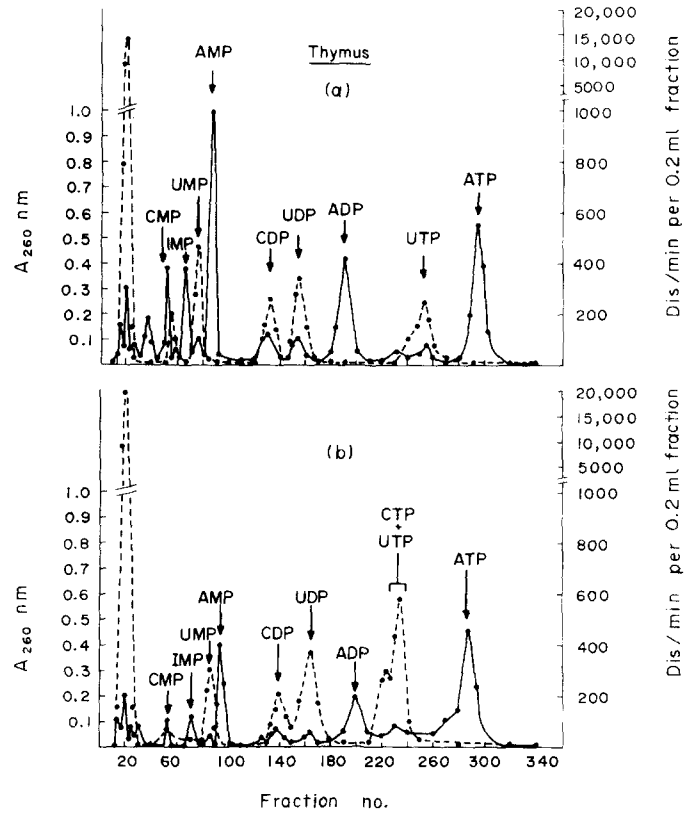

Fig. 2. Analysis of the acid-soluble fraction of rat thymus. (a) Rats received 60 μCi uridine[2-^{14}C] i.v. 15 min prior to sacrifice. Thymus was removed from three rats and dropped into liquid nitrogen for preparation of the acid-soluble fraction. (b) Three rats were injected i.v. each with IPAR (200 mg/kg) 30 min prior to the administration of 60 μCi UR[2-^{14}C] and sacrificed 15 min thereafter. Key: ● ——●, absorbance at 260 nm; ●- - - -●, cpm/1.2-ml fraction. Gradient conditions as in the legend of Fig. 1 except that 1.2-ml fractions were collected.

Table 2. Effects of IPAR on thymus pyrimidine nucleotides and on the incorporation of uridine[^{14}C] into these nucleotides*

Components	Control		IPAR-treated		Specific activity†		Ratio of specific activity† (IPAR-treated/control)
	$A_{260\,g}$	cpm/g ($\times 10^{-4}$)	$A_{260\,g}$	cpm/g ($\times 10^{-4}$)	Control ($\times 10^{-4}$)	IPAR-treated ($\times 10^{-4}$)	
Total acid-soluble extract	56.0	44.60	52.0	52.10	0.79	1.01	1.28
Total ribonucleosides	4.30	35.00	4.60	36.00	8.20	7.82	0.95
Pyrimidine ribonucleotides							
CMP	0.88	0.26	0.52	0.13	0.30	0.25	0.83
UMP	0.84	1.20	0.30	0.87	1.41	2.90	2.05
CDP	2.60	1.16	0.60	1.25	0.45	2.08	4.62
UDP	1.18	1.53	0.42	2.88	1.30	6.86	5.27
CTP + UTP	4.00	2.30	3.80	6.50	0.58	1.71	2.94
Total pyrimidine nucleotides	9.50	6.45	5.64	11.63	0.68	2.10	3.10

* ^{14}C-labeled and unlabeled fractions eluted from the DEAE-cellulose, type DE-52, columns in the area of pyrimidine ribonucleosides, mono-, di-, and triphosphates were pooled, concentrated and the 280/260 nm absorbance ratio and total cpm content were determined. These values represent the average of two experiments (pooled tissue from three rats/experiment).

† Specific activity is defined as cpm/A_{260}.

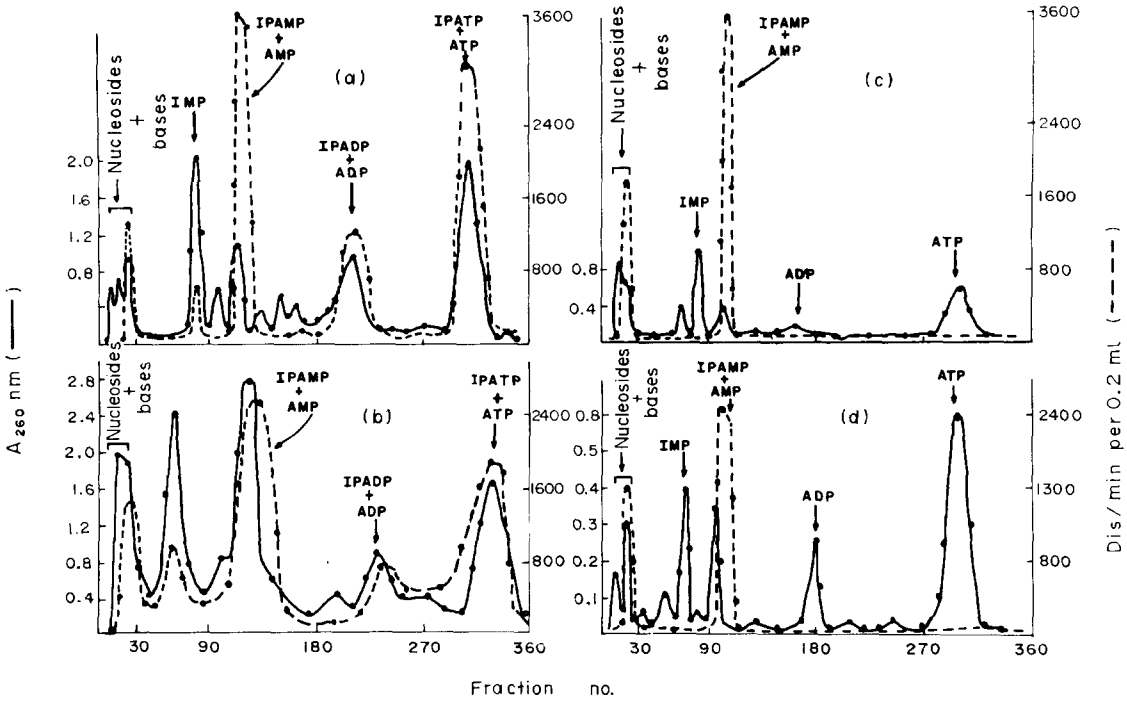

Fig. 3. Chromatographic separation of rat tissue acid-soluble fractions 4 hr after an i.v. administration of 15 μCi IPAR[8-^{14}C] to each of eight rats: a, b, c and d are chromatographic profiles of liver, small intestine, spleen and thymus respectively. Tissues from two treated rats are pooled for each profile. Gradient conditions are as outlined in Fig. 1 except that 1.2-ml fractions were collected.

ribonucleoside peaks (fractions 10–30) were identified as AR and IPAR. Five radioactive ribonucleotide peaks were eluted from the calibrated DEAE-cellulose, type DE-52, column in positions corresponding to IMP, AMP, GMP, ADP and ATP. At 30 min after IPAR[8-^{14}C] administration (data not shown), in addition to the nucleoside peak, only one major ^{14}C-labeled nucleotide peak was eluted and, in contrast, it was in the general area of AMP. Figure 3b shows the chromatographic profile of rat small intestine after IPAR[8-^{14}C] administration. The peaks of radioactivity (Fig. 3b) generally corresponded to those obtained in Fig. 3a. In rat spleen (Fig. 3c) and thymus extract (Fig. 3d), only one ^{14}C-labeled peak was eluted (in the general area of AMP) with no detectable formation of ^{14}C-labeled nucleosides, or di- and triphosphates.

Although Figs. 1 and 2 indicate the usual positions of nucleotides and nucleosides, final identification of ^{14}C-labeled fractions cannot be based solely on relative order of elution from the DEAE-cellulose columns since IPAMP + AMP co-chromatographed as a single peak. For this reason we expected that IPADP + ADP, and IPATP + ATP might behave in similar fashions. To confirm the extent of IPAR metabolism, fractions eluted in the general areas of AMP and ATP were concentrated, enzymatically digested to the nucleosides, and re-chromatographed on Silica gel for separation of AR from IPAR. After localization of the u.v. absorbent spots and extraction from the gel, the absorbance ratios at various wavelengths were determined (Table 3). The data in Table 3 show that the enzymatically digested nucleotides possess absorbance ratios which are similar to their authentic counterparts.

The amounts of IPAR and its metabolites found in rat liver, small intestine, spleen and thymus are summarized in Table 4. At 4 hr after the administration of IPAR[8-^{14}C], IPAR was phosphorylated to the 5′-mono-, di-, and triphosphate in liver and small intestine. At this time, only 5′-monophosphate could be identified in spleen and thymus. The results also indicated that the extent of IPAR conversion to AR was greater in small intestine than that in liver.

Table 3. Characterization of IPAR metabolites in rat liver*

Compound	Absorbance ratios at pH 12.0		
	290/260	280/260	250/260
IPAR-authentic	0.31	0.83	0.52
AR-authentic	0	0.14	0.76
Monophosphate			
IPAR	0.28	0.75	0.48
AR	0	0.18	0.86
Triphosphate			
IPAR	0.30	0.72	0.52
AR	0	0.18	0.86

* ^{14}C-labeled fractions eluted from the DEAE-cellulose, type DE-52, column (Fig. 3) in the area of mono- and triphosphate were concentrated and enzymatically digested to the riboside level and re-chromatographed on Silica gel for identification and separation of AR from IPAR ribonucleosides. Spots were extracted with 0.01 N NaOH and concentrated, and the absorbance ratios were determined using a DW-2 spectrophotometer with 0.05 O.D. units as full scale.

Table 4. Distribution *in vivo* of IPAR[8-^{14}C] in rat liver, small intestine, spleen and thymus 4 hr after an i.v. administration of the labeled drug*

	Radioactivity (cpm/g tissues, $\times 10^{-5}$)			
	Liver	Small intestine	Spleen	Thymus
Acid-soluble (total)	5.7	3.3	2.2	0.88
DE-52 column fraction†				
IPAR	0.40	0.10	0.22	0.07
AR	0.23	0.33	0.04	0.03
IPAMP	1.80	0.66	1.65	0.70
AMP	0.80	0.66	0.16	0.02
IPADP	0.46	0.20	ND‡	ND
ADP	0.57	0.46	ND	ND
IPATP	0.74	0.23	ND	ND
ATP	0.57	0.56	ND	ND
%Recovery	97	97	94	93

* IPAR[8-^{14}C] (15 μCi) was administered i.v. to each of two rats. At 240 min after the administration of the drug, rats were anesthetized and tissues removed quickly and pooled for preparation of acid-soluble fraction.

† Quantitation of ribonucleosides and ribonucleotides was made by t.l.c. chromatography of pooled and enzymatically digested samples eluted from the DEAE-cellulose, type DE-52, column.

‡ ND = not detectable.

Incorporation of IPAR[8-^{14}C] into nucleic acids and protein

Incorporation of IPAR[8-^{14}C] into macromolecules of rat tissues 4 hr after drug administration was investigated. DNA, RNA and protein fractions were isolated from each tissue and counted for ^{14}C-radioactivity. Of the four tissues studied, liver and small intestine were the only ones that showed incorporation of ^{14}C-label into RNA. No significant incorporation of IPAR[8-^{14}C] into the DNA and protein was detected in any of the tissues investigated. The results are summarized in Table 5.

To eliminate the possibility that the radioactivity liberated from RNA after its enzymatic digestion may have resulted from the release of non-covalently bound nucleotides, a portion of the labeled RNA fraction was gel filtered on a Bio-Gel and fractions were counted and enzymatically digested. The present evidence indicates that IPAR[8-^{14}C] was incorporated into RNA of liver and small intestine: (1) radioactivity of the gel-filtered materials was directly associated with the eluted RNA; (2) chromatographic separations using TLC and paper of the enzymatically digested RNA revealed that only two labeled components were released and these had R_f values identical with AMP and IPAMP. Furthermore, when the digestion was carried to the nucleoside levels, the two labeled components were identified as AR and IPAR; and (3) the 280/260 nm absorbance ratios of the released labeled components with an R_f value corresponding to AMP and IPAMP were 0.17 and 0.78 respectively. In contrast, enzymatic digestion of spleen RNA released only a single labeled nucleoside identified as AR with no detectable release of IPAR.

Table 5. Uptake and incorporation of IPAR[8-^{14}C] into tissues acid-soluble and -insoluble fractions 4 hr after an i.v. administration of the drug*

	Radioactivity (cpm/g Tissues)				
	RNA				
	Total	AMP [8-^{14}C]	IPAMP [8-^{14}C]	DNA†	Protein†
Liver	19,000	9,000	9,000	<200	<200
Small Intestine	12,000	6,000	5,000	<200	<200
Thymus	1,000	1,000	<50	<50	<50
Spleen	1,000	1,000	<50	<50	<50
R_f					
Silica gel		0.88	0.52		
3MM		0.32	0.89		

* Acid-soluble fractions were prepared (see Methods) and each fraction was counted for total radioactivity. Liver (15 g), small intestine (6 g), thymus (1.1 g) and spleen (1.2 g) were used for preparation of the acid-insoluble fraction. DNA, RNA and protein were isolated from the acid-insoluble fractions. ^{14}C-labeled RNA was then enzymatically digested, and incorporated label was identified on Silica gel (see Ref. 13) and on 3 MM paper.

† Due to the low level of incorporation into these fractions, identification of the incorporated label was not attempted.

A schematic representation for the anabolic and catabolic pathways of IPAR is outlined in Fig. 4.

DISCUSSION

As indicated in Fig. 4, IPAR may be phosphorylated to the 5'-mono-, di-, and triphosphates and incorporated into RNA. In addition, the drug may be

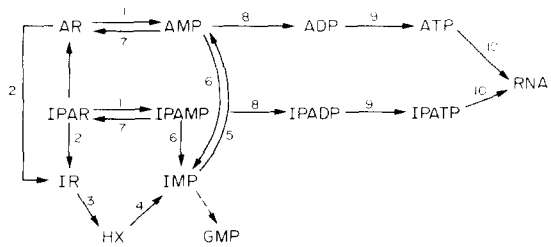

Fig. 4. Schematic representation of the metabolism of IPAR and AR. (1) adenosine kinase (ATP: adenosine 5'-phosphotransferase) (EC 2.7.1.20); (2) adenosine deaminase (adenosine aminohydrolase) (EC 3.5.4.2); (3) nucleoside phosphorylase (orthophosphate ribosyltransferase) (EC 2.4.2.1); (4) hypoxanthine phosphoribosyl-transferase (IMP: pyrophosphate phosphoribosyltransferase) (EC 2.4.2.8); (5) adenylosuccinate synthetase and adenylosuccinate lyase [IMP L-asparatate ligase (GDP)] (EC 6.3.4.4), and adenylosuccinate AMP-lyase (EC 4.3.2.2), respectively; (6) adenylate deaminase (AMP-aminohydrolase) (EC 3.2.2.4); (7) phosphatase (orthophosphoric monoester phosphohydrolase) (EC 3.1.3.1); (8) adenylate kinase (ATP: AMP phosphotransferase) (EC 2.7.4.3); (9) adenosine diphosphate kinase (ATP:ADP phosphotransferase) (EC 2.7.4.6); (10) RNA polymerase (ATP-RNA nucleotidyl-transferase) (EC 2.7.7.6).

catabolized to form various adenosine nucleotides with subsequent incorporation into RNA. It is evident that tissue selectivity of IPAR may depend on the rate of catabolism and anabolism of this drug in different tissues. Enzyme preparations from human or chicken bone marrow, pea seedlings, and tobacco pitch tissue cultures have been shown to convert IPAR to N^6-(3-hydroxy-3-methyl-butyl)-adenosine and hypoxanthine [21, 22] or to inosine [23]. Tobacco leaves also contain an enzyme that hydrolyzes IPAR to adenosine [24]. In humans, however, a large amount of IPAR[8-^{14}C] was excreted in the uridine as non-ultraviolet absorbing compounds [25].

The current investigation demonstrates that in rat tissues both anabolic and catabolic pathways for IPAR are operable, since both labeled drug and labeled purine nucleosides and nucleotides were found in the acid-soluble fraction after administration of IPAR[8-^{14}C] (Fig. 3 and Table 4). These results, however, do not discriminate among any of the several possible pathways as to the most probable pathway to the formation of IMP and AMP from IPAR. The evidence presented here also demonstrates the conversion of IPAR to purine nucleosides. With spleen and thymus, our results further confirm those of Divekar *et al.* [7], Hacker [8] and McLennan and Pater [9] in which IPAR[8-^{14}C] was phosphorylated to the 5'-monophosphate only in cells in culture with no evidence of drug incorporation into RNA. The possibility remains of course that incorporation into RNA might occur at a significant level in these tissues were treatment to be extended beyond the 4-hr period used in the present study. In rat liver and small intestine, the labeled drug was rapidly phosphorylated up to the triphosphate, and incorporation into RNA was quite extensive by 4 hr. Furthermore, the results in Table 5 indicate that not only was the amount of IPAR incorporated into RNA of rat liver greater than that incorporated into small intestine at this time, but also that the rate of IPAR catabolism in small intestine was over 2-fold greater.

The results summarized in Table 2 demonstrate that in rat thymus the drug produced about a 50 per cent decrease in the amount of pyrimidine nucleotides found in the acid-soluble fraction. As the result of this decrease, a 2- to 5-fold increase in the specific activity of pyrimidine nucleotides after administration of UR[^{14}C] was achieved. These results suggest that the observed stimulation of precursor incorporation into thymus RNA [11] may be the result of inhibition of the *de novo* pathway of pyrimidine nucleotide biosynthesis by the drug and/or its metabolites.

Results obtained in this investigation suggest that the pronounced inhibition of precursor incorporation into liver protein [13] may possibly be related, in part, to the reduction in the ATP concentration by IPAR (from 2.35 to 0.70 mole/g, Table 1) at 30 min. This drop coincided with the maximum inhibition of incorporation of [^{14}C]phenylalanine into liver protein (95 per cent) and the accumulation of about 2.0 μmoles IPAMP/g. The change in the adenosine pool produced by the drug also resulted in a downward shift in the adenylate energy charge [26]. In sient, returning to about control values within 4 hr after drug administration.

This study provides evidence for the phosphorylation of IPAR into 5'-mono, di-, and triphosphate nucleotides and for the incorporation of this analog into RNA of rat liver and small intestine. In contrast to these tissues, the drug was phosphorylated only to the 5'-monophosphate level in spleen and thymus and less than 1 per cent of IPAR was converted to AR in these tissues. These differences in metabolism reflect differences in the biochemical make-up of various normal tissues and may provide some bases for an understanding of the selective cytotoxicity of this agent.

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