Pyrimidine Nucleotide Synthesis in the Normal and Hypertrophying Rat Heart

RELATIVE IMPORTANCE OF THE DE NOVO AND "SALVAGE" PATHWAYS

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ABSTRACT

Radioactive orotic acid incorporation into RNA (de novo pathway of pyrimidine nucleotide synthesis) was considerably lower for rat heart than for rat liver in vivo and in vitro. $^3$H uridine ("salvage" pathway) was incorporated into heart RNA to a greater extent than $^3$H orotic acid, and the labeling with uridine in the heart exceeded that in the liver. Extracts of heart showed little enzymatic conversion of orotic acid to pyrimidine nucleotides in the presence of ATP and ribose-5-phosphate, a condition under which there was good activity in other tissues such as the liver, spleen, and kidney. Addition of phosphoribosylpyrophosphate (PRPP) markedly enhanced orotic acid conversion though it still remained less for heart than for other tissues. These findings indicated that PRPP synthetase activity was low in the heart. Uridine kinase activity in the heart was similar to that in the liver. Uridine kinase appeared to be rate limiting in the "salvage" pathway. Aortic constriction produced an increase in uridine kinase activity at 24 hours with a peak at 2 to 6 days (50 to 60% stimulation) after operation, while uridylic kinase, uridine phosphorylase, and orotidine monophosphate pyrophosphorylase activities remained unchanged. The "salvage" pathway appears to play an important role in pyrimidine nucleotide synthesis in the heart, and uridine kinase may be regulatory in this pathway during cardiac hypertrophy.

ADDITIONAL KEY WORDS

uridine kinase uridylic kinase
uridine phosphorylase orotidine monophosphate pyrophosphorylase
phosphoribosylpyrophosphate synthetase labeling of heart RNA
uridine and orotic acid utilization by heart constriction of aorta
enzymes in liver and skeletal muscle

Pyrimidine nucleotides are synthesized in several bacterial species, in yeast, and in a number of mammalian tissues through the de novo pathway, which consists of a series of reactions in which orotic acid is an intermediate (1-3). The so-called "salvage" pathway (3-5) is available for reutilization of the products of nucleic acid degradation. The salvage pathway appears to play an important role in pyrimidine nucleotide synthesis in certain bacterial species (4) and in various mammalian tumors (7, 8). Although there is evidence for activity of the salvage pathway in several normal mammalian tissues, its role as a predominant pathway for the synthesis of pyrimidine nucleotides by these tissues has not been established.

A diagram of the de novo and salvage pathways of pyrimidine synthesis is shown in Figure 1. After the formation of the pyrimidine base by a series of reactions in the de novo pathway, orotic acid is converted to OMP in the presence of OMP pyrophosphorylase. OMP is decarboxylated to UMP before...
further phosphorylation of UMP to UDP and UTP in the presence of uridylic kinase. In the salvage pathway, uracil may be converted to uridine by the enzyme, uridine phosphorylase. Uridine kinase catalyzes the formation of UMP from uridine. Uracil is also directly condensed with PRPP to form UMP in a reaction catalyzed by uracil phosphoribosyltransferase. However, this pathway is believed not to be active in mammalian tissues. During a series of experiments on the synthesis of nucleic acids, we found that orotic acid was a poor precursor for heart RNA, while it was incorporated very well into the RNA of other tissues such as the liver. On the other hand, labeling of RNA with uridine was relatively high in the heart. Although differences in transport across cell membranes and in intracellular pool sizes could account for this observation, it seemed equally plausible that the activities of various enzymes involved in the de novo and salvage pathways in the heart might differ from those in other tissues.

Therefore, we undertook a study of several of the enzymes in the metabolic pathways of pyrimidine nucleotide synthesis and compared their activities in the heart with activities in other tissues. There was considerable enzymatic activity in the salvage pathway of pyrimidine nucleotide synthesis and low conversion of orotic acid to pyrimidine nucleotides by heart extracts under conditions in which there was active conversion by extracts from other tissues. These findings support the concept that the heart may utilize the salvage pathway to a large extent relative to the de novo one for the synthesis of pyrimidine nucleotides under normal conditions. Furthermore, there was a stimulation of the salvage pathway after aortic constriction as manifested by an increase in uridine kinase activity while enzymatic activity in the de novo pathway remained unchanged.

Methods and Materials

ANIMALS

Male albino rats were obtained from the Charles River Breeding Laboratories and maintained on Purina chow ad libitum. Aortic constriction and sham operations were carried out as previously described (10) on animals that weighed 180 to 220 g. In each experiment, operations were carried out on several sets of
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animals over a period of several days. These animals were all killed on the same day so that preparations and assays of all enzymes in each experiment could be performed simultaneously. Most experiments contained hearts from animals 2 hours and 1, 2, and 6 days after operation. A few experiments included animals 13 days after operation. Chloral hydrate (60 to 70 mg per animal) was given intraperitoneally for anesthesia. In some experiments in which the various enzymatic activities were characterized, animals up to 350 g in weight were used. All animals were killed by decapitation, and tissues were rapidly removed and kept in containers on ice until sliced or homogenized.

RADIOACTIVE LABELING OF RNA WITH \( ^{3}H \) URIDINE, \( ^{3}H \) UMP, \( ^{3}H \) URACIL AND \( ^{3}H \) OROTIC ACID

For in-vitro experiments, tissue slices weighing approximately 200 to 400 mg were obtained with a Stadie-Riggs tissue slicer and were kept briefly in ice-cold saline until they were added to 10 ml of modified Krebs-Ringer bicarbonate medium, supplemented with amino acids, antibiotics and dextrose, to start the incubation. The incubation medium was prepared as follows: asparagine (2.5 mg), aspartic acid (3.5 mg), serine (15.4 mg), glutamic acid (9.1 mg), glycine (16.1 mg), alanine (21.4 mg), proline (13.9 mg), cysteine (3.0 mg), and tryptophan (5.0 mg) were added to 5.0 ml of Eagle's 100 X concentrated amino acid solution (Microbiological Associates, Inc.), and the pH was adjusted to 7.4 with NaOH. The amino acid mixture was diluted (1:100) with modified Krebs-Ringer bicarbonate (11). The following substances were added to 100 ml of this solution: glutamine (20.2 mg), streptomycin (20 mg), penicillin (5,000 units), polymyxin B sulfate (1 mg), and dextrose (100 mg). Fifty microcuries of \( ^{3}H \) uridine, \( ^{3}H \) UMP, or \( ^{3}H \) uracil or 100 \( \mu \)c of \( ^{3}H \) orotic acid were added to 100 ml of incubation medium. The final specific activity of these compounds in the medium was made the same by the addition of unlabeled uridine, UMP, or uracil.

The incubation was carried out at 37°C in a Dubnoff metabolic shaker gassed with a 95% \( O_2 \):5% \( CO_2 \) mixture and was terminated by removing the tissue slices, washing them briefly with saline and homogenizing them by hand in 2 ml of ice-cold 10% trichloroacetic acid. The sediment was separated by centrifugation and was washed twice with ice-cold 10% trichloroacetic acid. It was then left overnight in 95% ethanol-6% K acetate (12) prior to two further washes with 95% ethanol. The sediment was dried in a desiccator. A sample of about 25 mg of sediment was extracted with 5 ml 10% NaCl (13) at 100°C for 60 minutes. RNA was precipitated by making the extract 68% in ethanol, and subsequently was washed with ethanol, dried, and dissolved in water. The amount of RNA present was estimated by measuring the optical density at 260 m\( \mu \). One optical density unit was equivalent to 32.2 mg • ml\(^{-1} \) • cm\(^{-1} \) (13). A sample was counted in a Packard Tri-Carb liquid scintillation counter in scintillation fluid containing 0.5 g 2,5-diphenyloxazole (PPO) and 0.4 g 1,4-bis-(2-(5-phenyloxazolyl)) benzene (POPOP) in toluene:2-ethoxyethanol:2:1. Specific activity was expressed as counts • min\(^{-1} \) • mg\(^{-1} \) RNA.

For in-vivo experiments \( ^{3}H \) uridine, \( ^{3}H \) uracil, or \( ^{3}H \) orotic acid was injected intravenously in the tail vein and animals were killed 1 hour later. Organs were removed and tissue samples were handled as described above for the extraction of RNA and the measurement of its specific activity.

PREPARATION OF TISSUE EXTRACTS

Organs were removed, rinsed with homogenizing medium, blotted, weighed, and homogenized in three volumes of ice-cold 0.25M Tris-HCl, pH 7.4, in a Thomas 4228-B tissue homogenizer with
a motor-driven pestle. In experiments in which orotic acid conversion was measured, a solution containing 0.05M Tris, pH 7.4, or 0.02M Tris and 0.05M Na phosphate, pH 7.4 (14) was used for homogenization. The homogenate was centrifuged in a Beckman Model L Ultracentrifuge at 100,000 X g for 90 minutes, and the supernatant fluid was carefully pipetted and used as the "tissue extract."

**ENZYME ASSAYS**

All assays were carried out at 37°C. The reaction was started by the addition to the "tissue extract" and was terminated by adding 1/20th volume of 100% trichloroacetic acid (w/v) to the incubation medium. After centrifugation, a sample of the supernatant fluid was added to an appropriate group of "carriers" (uracil, uridine, UMP, UDP, and UTP) whose final concentration was approximately 10⁻³M; and 10-20 µl of each sample was applied to one of the chromatographic systems described below. In cases where paper-strip chromatography was utilized, spots were identified under ultraviolet light, and areas of the strip were cut out and counted directly in scintillation fluid containing 0.5 g% PPO and 0.04 g% POPOP in toluene in a Packard Tri-Carb liquid scintillation counter. Control samples without added extract were run routinely and subtracted as background. More than 95% of the radioactivity remained at the location of the substrate for all of the control samples. Enzymatic activity was expressed either as the percent of total counts on the chromatogram which were present in the product(s) of the reaction under analysis, or as the absolute quantities of the product(s) formed. All values expressed at a single time or concentration of enzyme activity were obtained under conditions in which there was relatively linear time and concentration dependence of the enzyme activity.

**URIDINE KINASE ASSAY**

The usual incubation was carried out by adding 0.5 to 0.1 ml of tissue extract to 0.5 ml of medium which contained 0.05M Tris-HCl, pH 7.8, 0.05M MgCl₂, 0.05M ATP, 5 x 10⁻⁶M uridine, and 5 µCi/ml of 'H uridine. After termination of the incubation, a sample was added to a mixture of unlabeled uracil, uridine, UMP, UDP, and UTP, and chromatography was carried out on strips of Whatman DE51 diethylaminoethyl cellulose paper in a descending system with a solvent containing 4N formic acid and 0.1N ammonium formate (15). A 4-hour period provided good separation of all the above carriers except for uracil and uridine which had the same Rₜ value (Table 1). Hence, it could not be determined with this system how much uridine was being converted to uracil. For separation of uracil and uridine, some samples also were chromatographed in the butanol system described below. Uridine kinase activity was determined from the total radioactivity in all uridine nucleotides at the end of incubation. Under the conditions and duration of our enzyme assay, no more than 15 to 20% of uridine was converted to uracil. This did not interfere with the linearity of uridine conversion to nucleotides. Also, under the conditions of the uridine kinase assay, the rate of backward conversion from UMP to uridine, as determined with 'H UMP, never exceeded about 10% of that going in the forward direction. Although the kinetics of enzyme activity in the crude extract were somewhat complex, the multiplicity of reactions did not seem to interfere with the linearity of uridine kinase activity.

**URIDYLATE KINASE ASSAY**

The activity of uridylate kinase in the tissue extract was usually measured by the addition of 0.1 ml of extract to 0.5 ml of medium which contained 0.05M Tris-HCl, pH 7.8, 0.05M MgCl₂, 0.0025M EGTA, 0.005M ATP, 0.05M UMP, and 5 µCi/ml of 'H UMP. Chromatography was carried out...
out on paper strips with the same system as that for uridine kinase, and uridylate kinase activity was expressed as UMP conversion to UDP plus UTP. With this assay, a small amount of radioactivity was also found in the uridine-uracil area; but under the conditions used, this was less than 20% of the total radioactivity and did not interfere with the linearity of the conversion of UMP to UDP plus UTP.

**Uridine Phosphorylase Assay**

The conditions of the spectrophotometric assay described by Razzell (16) were modified for an assay which utilized the conversion of radioactive uridine to uracil. The incubation was carried out in a medium which contained .005M sodium arsenate-acetate buffer pH 7.5, 10-3M uridine and 1 μC/ml of 3H uridine. The presence of arsenate makes this reaction irreversible since the arsenate combines with ribose formed from uridine. Separation of substrate and products was carried out either with ascending chromatography for 26 hours on thin-layer Eastman DEAE cellulose without fluorescent indicator or with descending chromatography for 15 hours on strips of Whatman 1 chromatography paper with a solvent system of water saturated n-butanol containing ammonium bicarbonate (17) (Table 1). Spots were identified under ultraviolet light. There was good separation of uracil and uridine from the nucleotides which remained at the origin. Areas of the thin-layer chromatography were scraped off and eluted with 1 ml of 0.1N HCl; 0.5 ml of the eluate was counted in scintillation fluid containing 0.04 g% POPOP and 0.5 g% PPO in toluene.

**Assays for Conversion of Orotic Acid**

The conversion of orotic acid to various products by tissue extract was measured essentially in one of two ways—either in the presence of PRPP or with ATP and ribose-5-phosphate added for the production of PRPP by enzymes in the extract. To measure the conversion of orotic acid in the presence of PRPP, 0.1 ml of extract was added to .05 ml of incubation medium containing 0.1M Tris-HCl, pH 7.4, 0.1M MgCl2, 5 X 10-5M orotic acid, 0.1M PRPP and 4.4 μC/ml of 3H orotic acid. In these experiments, the reaction was stopped with trichloroacetic acid as noted above. Descending chromatography was carried out for 16 hours on strips of Whatman DE81 diethylaminoethyl cellulose chromatography paper with an ammonium acetate-ethanol-H2O solvent system as described by Skold (19). Good resolution of orotic acid, uridine-uracil, and uridylate nucleotides was obtained (Table 1). No more than approximately 18% of the counts were in the area of dihydroorotic acid. Radioactivity was distributed in UDP, UTP, and uridine-uracil as well as UMP, and we elected to express the results as total percent conversion of orotic acid (100% minus % of total radioactivity remaining in orotic acid).

The assay system containing PRPP was considered to measure orotidine monophosphate (OMP) pyrophosphorylase. We were unable to obtain OMP decarboxylase in a purified form to be sure that the amount of this enzyme added with the tissue extract was in excess, but assume that it was since: (1) the conversion of orotic acid was linear over the time periods used, and it would be expected that this would not occur if OMP accumulated; and (2) most radioactivity was found in the area of UTP when ATP was added. The assay system in the presence of ATP and ribose-5-phosphate is a measure of endogenous PRPP plus PRPP formed during incubation. Since there was very little, if any, conversion of orotic acid to nucleotides for any of the tissues without either ATP and ribose-5-phosphate or PRPP, orotic acid conversion in the presence of endogenous PRPP plus PRPP formed during incubation is presented.

**Materials**

Uracid-5-3H (23 c/m mole), uridine-5-3H (28 c/m mole) and UMP-5-3H (0.5 c/m mole) were purchased from Schwarz Bioresearch. Orotic-5-3H acid (0.83 c/m mole) was obtained from New England Nuclear Corp. Uridine, UMP, UDP, UTP, uracil, d-ribosel-5-phosphate, d-ribose-3-phosphate and d-3-phosphoglyceric acid were obtained from the Sigma Chemical Company. Orotic acid was purchased from Eastman Chemicals. PRPP was purchased from Sigma Chemical Company and Mann Research Laboratories.

**Results**

Incorporation of labeled precursors into RNA in Vitro—Figure 2 (top) demonstrates the relative incorporation of radioactive orotic acid.
Labeling of RNA in vitro and in vivo. In-vitro labeling of RNA of tissue slices was carried out as described in Methods. The concentrations of the labeled substrates were as follows: orotic acid (1.2 x 10^-5 M); uridine (5.9 x 10^-5 M); UMP (5.6 x 10^-5 M); and uracil (5.9 x 10^-5 M). Specific activity was 0.83 c/mmole for each of these substances. For in-vivo labeling, animals were injected with 0.5 mc of 3H orotic acid or 0.25 mc of 3H uridine or 3H uracil. The absolute quantity of labeling with uridine or uracil was multiplied by two so as to make the original doses of radioactivity equivalent to that of 3H orotic acid.

Incorporation of Labeled Precursors into RNA in Vivo.—The relative incorporation of radioactive precursors into RNA in vivo (Fig. 2) reflected the pattern found in vitro. In vivo there was considerably more incorporation of orotic acid into RNA of liver than into that of heart. The uridine incorporation into RNA of liver was less than 1/3 of that of orotic acid incorporation. Labeling of heart RNA with uridine exceeded that for liver and was several times more than orotic acid labeling of heart RNA. As noted in the in-vitro studies, labeling with uracil was relatively low for both the heart and the liver.

Enzymes of Pyrimidine Synthesis.—Figure 3 shows a comparison of the activities of various enzymes of pyrimidine synthesis in extracts of heart tissue. The activities of uridylate kinase and uridine phosphorylase were quite high as compared to the activities of uridine kinase and OMP pyrophosphorylase. Their apparent K_m's were considerably lower than those of uridine kinase and OMP pyrophosphorylase. The maximum activities of uridine kinase and OMP pyrophosphorylase were similar.

Enzymes in the De Novo Pathway of Pyrimidine Nucleotide Synthesis.—Extracts of...
PYRIMIDINE NUCLEOTIDE SYNTHESIS IN HEART

TABLE 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>OMP pyrophosphorylase (mimoles • 0.1 ml⁻¹ • min⁻¹)</th>
<th>PRPP synthetase activity (mimoles • 0.1 ml⁻¹ • min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP and ribose-5-phosphate</td>
<td>PRPP</td>
</tr>
<tr>
<td>Heart</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>Liver</td>
<td>.00</td>
<td>.31</td>
</tr>
<tr>
<td>Kidney</td>
<td>.00</td>
<td>.15</td>
</tr>
<tr>
<td>Spleen</td>
<td>.00</td>
<td>.56</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>.00</td>
<td>.06</td>
</tr>
</tbody>
</table>

Assays were carried out as described in Methods.

both heart and skeletal muscle showed little activity in the conversion of orotic acid in the presence of ATP and ribose-5-phosphate without added PRPP (Table 2). Extracts of several other tissues showed relatively good orotic acid conversion under these conditions. There was little, if any, activity in any of the extracts without the addition of ATP and ribose-5-phosphate or PRPP. Orotic acid conversion by heart extract was enhanced markedly with the addition of PRPP. Nevertheless, the activity in heart was only one-ninth that in the liver even in the presence of PRPP.

The finding of low orotic acid conversion by the heart in the presence of ATP and ribose-5-phosphate suggests that there was low PRPP synthetase activity in the heart extract. To eliminate the possibilities of degradation of PRPP synthetase during tissue homogenization or instability of PRPP in the presence of heart extract during incubation, the following experiments were performed: (1) Mixtures of heart and liver tissue were homogenized together and were found to yield additive activities of the extract (Fig. 4); and (2) PRPP was preincubated with heart extract before its use in measuring orotic acid conversion by liver extract, and no evidence was found for inactivation of PRPP by the heart extract (Fig. 5). It was also considered that ATP might be destroyed in the assay system by heart extract. However, examination of a chromatogram of a sample of the postincubation medium under ultraviolet light showed no evidence for destruction of ATP.

Figure 6 shows substrate concentration curves and Lineweaver-Burk plots for OMP pyrophosphorylase activity in heart extracts. The apparent Km for orotic acid as substrate was approximately 1.8 \times 10⁻⁵M, similar to that reported by Lieberman et al. for yeast OMP pyrophosphorylase (19). However, the apparent Km for PRPP as substrate (approximately 10⁻⁴M) was considerably higher than that reported for yeast OMP pyrophosphorylase (18). The apparent Km for PRPP for liver extract was 0.7 \times 10⁻⁴M, similar to that for the

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Orotic acid conversion by liver extract after preincubation of PRPP with and without heart extract. Heart extract or H2O as a control (0.1 ml) were preincubated for 15 minutes at 37° C in 0.9 ml of medium containing .02M Tris-HCl, pH 7.4, .02M MgCl2, .03M phosphoglyceric acid, 0.2M Na2HPO4, .01M ATP, .01M ribose-5-phosphate, and varying amounts of PRPP. After the 15-minute preincubation period, the mixture was placed in a boiling water bath for 1 minute, then cooled on ice. This procedure destroyed all heart OMP pyrophosphorylase activity. The mixture was incubated for a second time for 15 minutes at 37° C after the addition of .03 ml of liver extract and 0.1 ml of 5 x 10^-5 M orotic acid (final concentration and specific activity were 5 x 10^-5 M and 4.4 μC/ml, respectively). Preincubation of PRPP with heart extract (closed circles), preincubation of PRPP without heart extract (open circles), orotic acid conversion by liver without preincubation or boiling (open squares).

The reason for the requirement for high concentrations of PRPP for enzyme saturation in the crude extract was unclear.

Uridine kinase activity in heart extracts was relatively low compared with the activities of uridylic acid and uridine phosphorylase (Fig. 3). This is consistent with the impression that uridine kinase is rate-limiting in the salvage pathway for the synthesis of uridine nucleotides (20-22). Extracts of various tissues were compared for uridine kinase activity, and it was found that the activity in heart was similar to that in liver and somewhat higher than that in skeletal muscle, although it was lower than the activity in other tissues such as the kidney and spleen (Table 2).

Figure 7 shows substrate concentration curves and Lineweaver-Burk plots for uridine kinase activity of the heart extract. The Km for uridine as substrate was 3.4 x 10^-5 M, and the Km for ATP as substrate was 2.7 x 10^-5 M. Skold previously reported the Km for uridine for uridine kinase activity in Ehrlich ascites cells to be 4.8 x 10^-5 M and the Km for ATP to be 5 x 10^-4 M (23).

Uridine kinase activity has been found to be subject to end product inhibition by UTP and CTP in mouse mast cell neoplasm, human epidermoid carcinoma, and Novikoff ascites rat tumor (22, 24). The effect of UTP, CTP and ATP on uridine kinase activity of heart extracts was tested. While there was little, if any, effect of GTP on enzymatic activity, both UTP and CTP produced marked inhibition of uridine kinase activity (Fig. 8).

Uridylate Kinase Activity.—When measuring uridine kinase activity, we found that the distribution of radioactivity in UMP, UDP, and UTP was at times quite variable and unpredictable even though the sum of the counts in these three nucleotides remained unchanged. Further studies showed that in the presence of EGTA, there was an increase in labeling of UTP and UDP and a decrease in labeling of UMP (Fig. 9A and B). It was of interest that this pattern could be reversed with the bulk of labeling in UMP, and little labeling of UDP and UTP when high concentrations of calcium were added to the incubation medium (Fig. 9B). Hence, it appeared that some substance in the tissue extract, which could be chelated, was inhibitory for uridylic acid kinase activity. Since relatively large amounts of calcium were necessary to reverse the EGTA effect, it was uncertain whether calcium was the inhibitor. EGTA was added routinely in assays for uridylic acid kinase activity. Under these cond
Effect of Aortic Constriction on Activities of Enzymes of Pyrimidine Synthesis—The activities of uridine kinase, uridylate kinase, uridine phosphorylase, and OMP pyrophosphorylase were measured in hearts at various times after aortic constriction. The results of this study are shown in Table 3 and Figure 10. There was a significant increase in the uridine kinase activity in heart extracts at 24 hours after aortic constriction, and this reached a peak level of 50 to 60% above controls between 2 and 6 days after operation. There was no
Discussion

These studies show little incorporation of orotic acid as compared to that of uridine into heart RNA. Pool size, compartmentalization, and membrane transport, as well as enzyme activities of uridylyl kinase, uridine phosphorylase, or OMP pyrophosphorylase after aortic constriction.

increase in uridine kinase activity at 2 hours after constriction. Since animals were still subdued at 2 hours when given intraperitoneal chloral hydrate, some experiments were carried out with animals given ether anesthesia, and there was still no change in uridine kinase activity at 2 hours after constriction. In contrast to the increase in uridine kinase activity, there was no significant increase in uridine kinase activity at 2 hours after constriction.
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FIGURE 8

Effect of GTP, UTP and CTP on enzyme activity of heart extract. Assays were carried out as described in Methods at various concentrations of GTP, UTP and CTP. The concentrations of orotidine and ATP were 5 x 10^-5 M and .005M, respectively. UTP (solid circles); CTP (open circles); GTP (open squares).

Enzyme Activities in Heart after Aortic Constriction

<table>
<thead>
<tr>
<th></th>
<th>2 hours</th>
<th>24 hours</th>
<th>2 days</th>
<th>6 days</th>
<th>12 days</th>
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<tr>
<td>Uridine kinase</td>
<td>0.220 ± 0.025 (3)</td>
<td>0.201 ± 0.012 (3)</td>
<td>0.171 ± 0.014 (3)</td>
<td>0.133 ± 0.031 (3)</td>
<td>0.257 (1)</td>
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<tr>
<td>Uridylate kinase</td>
<td>0.368 ± 0.025 (4)</td>
<td>0.385 ± 0.032 (4)*</td>
<td>0.364 ± 0.038 (4)*</td>
<td>0.322 ± 0.061 (3)*</td>
<td>0.369 ± 0.035 (2)</td>
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<tr>
<td>Uridine phosphorlyase</td>
<td>46.4 ± 4.8 (3)</td>
<td>67.0 ± 10.3 (4)</td>
<td>88.2 ± 9.1 (5)</td>
<td>88.2 ± 9.2 (6)</td>
<td>68.9 ± 0.8 (2)</td>
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<td>OMP pyrophosphorylase</td>
<td>4.25 ± 0.30 (4)</td>
<td>4.16 ± 0.50 (4)</td>
<td>4.38 ± 0.76 (4)</td>
<td>4.88 ± 0.71 (5)</td>
<td>4.40 (1)</td>
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<tr>
<td>Constriction</td>
<td>3.95 ± 0.45 (4)</td>
<td>4.18 ± 0.36 (6)</td>
<td>4.66 ± 0.64 (5)</td>
<td>4.94 ± 0.84 (3)</td>
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<td>Sham</td>
<td>0.314 ± 0.015 (5)</td>
<td>0.306 ± 0.008 (4)</td>
<td>0.287 ± 0.028 (4)</td>
<td>0.291 ± 0.033 (4)</td>
<td>0.314 (1)</td>
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<td>OMP pyrophosphorylase</td>
<td>0.334 ± 0.026 (6)</td>
<td>0.277 ± 0.017 (5)</td>
<td>0.355 ± 0.031 (5)</td>
<td>0.394 ± 0.073 (4)</td>
<td>0.397 ± 0.018 (2)</td>
</tr>
</tbody>
</table>

Mean values and standard errors of enzyme activities are expressed as nmol of substrate converted per 0.1 ml of extract per minute. The numbers of sets of animals studied are in parentheses. Each set contained hearts pooled from three to five animals.

*0.05 > P > 0.02; †0.10 > P > 0.05. All other differences between sham-operated animals and animals with aortic constriction were nonsignificant.
similar observation for rat heart and skeletal muscle was made previously by Hurlbert and Reichard (25). However, they did not assess the effect of added PRPP in their assay system. Our experiments showed marked stimulation of orotic acid conversion by heart extract with the addition of PRPP and suggested that the primary limitation in this reaction resulted from low PRPP synthetase activity in the heart extract. We found no evidence for destruction of PRPP synthetase during preparation of the extract or for instability of PRPP in enzyme assays which utilized heart extract. The low orotic acid conversion in the presence of ATP and ribose-5-phosphate was not specific for rat heart since relatively low activity was found also in extracts from mouse or guinea pig heart (unpublished data.) Whether or not the low labeling of RNA with orotic acid is a function of relatively low PRPP in heart tissue remains to be established.

The enzyme data combined with the relatively high labeling of heart RNA with "H uridine suggest an important role for the salvage pathway in the synthesis of pyrimidine nucleotides in the heart. An exact quantitative assessment of the relative importance of the de novo and salvage pathways in the intact heart cannot be derived from the present data since there is no information about the relative transport of precursors or pool sizes within the tissues.

The regulation of the enzymes of the de novo and salvage pathways of pyrimidine
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nucleotide synthesis is of interest. Feedback inhibition has been studied extensively for these enzymes (22-24, 26-28). It has been demonstrated in systems other than the heart that uridine kinase activity is regulated by UTP and CTP (22-24). Carbamyl-aspartate transferase, carbamyl-phosphate synthetase, and OMP decarboxylase, enzymes in the de novo pathway, are also subject to feedback inhibition (26-28). PRPP synthetase is subject to inhibition by various compounds including ADP, CTP, UTP, GDP, 1,3-diphosphoglyceric acid, and tryptophan (29, 30). It appears from the present studies that heart uridine kinase activity may be regulated by UTP and CTP. The regulation of the other enzymes has not been studied for the heart.

Aortic constriction produced a stimulation of uridine kinase activity while uridine phosphorylase, uridylate kinase, and OMP pyrophosphorylase activities remained unaltered. At present we are investigating the effect of constriction on PRPP synthetase activity. There is no information available on the effect of aortic constriction on the enzymes in the earlier portion of the de novo pathway. Koide and Rabinowitz reported a 50% increase in total uridine nucleotides at 48 hours after aortic constriction in the rat (31), and an increase in uridine kinase may regulate the increase in nucleotides.

The apparent selective stimulation of uridine kinase activity in the hypertrophying heart resembles the response seen in phytohemagglutin-induced RNA synthesis in lymphocytes (32). On the other hand, during liver regeneration, there is a marked increase in the activities of enzymes of both the de novo and salvage pathways (17) and, in particular, an early increase in OMP pyrophosphorylase (33).

If the salvage pathway does play an important role in pyrimidine nucleotide synthesis in the heart, the origin of the substrate for this pathway is not clear at present. Despite high uridine phosphorylase activity, uracil was incorporated very poorly into heart RNA as well as into that of other rat tissues. Canellakis has explained the failure to incorporate uracil into liver RNA as being due to high catabolic activity for uracil in the liver (34), and this explanation, although untested, may also apply for the heart. Our experiments indicate that uridine is more favorable than uracil as a substrate for the formation of pyrimidine nucleotides by the heart. Either dietary uridine or endogenous uridine formed from uridine nucleotide pools in other tissues could be utilized by the heart. There is at present no supporting evidence for the speculation that the heart may derive uridine from other organs. However, the postulation that one tissue may be dependent upon a nucleoside produced in another has a precedent in experiments done by Lajtha and Vane in which they found that bone marrow is dependent upon the liver for its supply of purines (35).

Skeletal muscle resembled the heart in its conversion activities for uridine kinase and orotic acid. Further studies are needed to determine the degree of similarity between heart and skeletal muscle in pyrimidine metabolism.

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References


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