EVIDENCE FOR THE CONTROL OF PYRIMIDINE BIOSYNTHESIS IN TISSUE MINCES BY PURINES*

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SUMMARY: The rate of incorporation of $NaH^{14}CO_3$ into orotic acid in minces of chick oviduct and rat mammary gland was found to be markedly inhibited by the addition of adenosine or guanosine to the reaction mixture. By employing various ^{14}C-labeled precursors intermediate in the incorporation of bicarbonate into orotic acid, the site of inhibition by purines of the de novo biosynthesis of pyrimidines was determined to be the initial reaction in the sequence, that catalyzed by carbamoylphosphate synthetase.

Following the recent observation in our laboratory that uridine or its metabolites control the rate of pyrimidine biosynthesis in slices of several rat tissues through end-product inhibition of carbamoylphosphate synthetase (1), we sought to determine what influence purines might have on the de novo biosynthesis of pyrimidines under similar conditions. The effects of purine and pyrimidine nucleosides on the rate of incorporation of ^{14}C-labeled precursors into orotic acid were compared in experiments employing tissue minces of the estrogen-stimulated chick oviduct and the lactating rat mammary gland. Guanosine was found to inhibit the rate of incorporation of $NaH^{14}CO_3$ into orotic acid in each of these tissues with equal or greater effectiveness than uridine (TABLE I). Since 6-azauridine was employed to block the further metabolism of orotic acid in the experiments with chick oviduct, the alternative possibility was examined that guanosine or uridine was acting as an antagonist of 6-azauridine, thereby permitting the conversion of orotic acid to UMP rather than inhibiting its synthesis de novo. This alternative interpretation was eliminated following direct measurements of the effect of

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TABLE I

SITE OF INHIBITION OF PYRIMIDINE BIOSYNTHESIS BY PURINE AND PYRIMIDINE NUCLEOSIDES

	Incorporation of Precursor into Orotic Acid	
Precursor: $NaH^{14}CO_3$ Additions:	chick oviduct (% Control)	rat mammary gland (% Control)
None (Control)	100	100
Uridine, 5mM	10	53
Guanosine, 5mM	5	9
Precursor: ^{14}C-CP Additions:		
None (Control)	100	100
Uridine	94	97
Guanosine	92	105
Precursor: ^{14}C-CA Additions:		
None (Control)	100	100
Uridine	101	105
Guanosine	98	105

Tissue minces weighing 500 mg were incubated in Krebs Improved Ringer II Solution (2) with the isotopically labeled precursor indicated above, and the extent of incorporation of label into orotic acid was determined following isolation of the ^{14}C-orotate synthesized during the incubation period by co-crystallization with carrier orotate as described in a previous communication (1). In the experiments with chick oviduct, the tissue was excised following 1-3 days of treatment with diethylstilbestrol (5 mg subcutaneously daily), the incubation medium (10 ml) was adjusted to 7.5 mM in 6-azauridine and 7.5 mM in bicarbonate (37.5 μCi where $NaH^{14}CO_3$ was employed), and the reaction was allowed to proceed for 3 hr at 41°C. ^{14}C-carbamoylphosphate (^{14}CP) and ureido-^{14}C-carbamoylaspartate (^{14}C-CA) were employed at reaction concentrations of 5mM (1.3 μCi) and 2.5 mM (10 μCi), respectively, where indicated. The rates of incorporation under control conditions averaged 47, 63, and 318 nanomoles/gm tissue · hr for $NaH^{14}CO_3$, ^{14}C-CP, and ^{14}C-CA, respectively. In the experiments with rat mammary glands, the glands were excised at various times during lactation, the incubation medium (20 ml) was adjusted to 15 mM in bicarbonate (50 μCi where $NaH^{14}CO_3$ was employed), and the reaction allowed to proceed for 4 hr at 37.5°C. ^{14}C-CP (1.5 μCi) and ^{14}C-CA (1.1 μCi) were employed at reaction concentrations of 5mM each, where indicated. The rates of incorporation under control conditions averaged 16, 66, and 782 nanomoles/gm tissue · hr for $NaH^{14}CO_3$, ^{14}C-CP, and ^{14}C-CA, respectively.

guanosine and uridine on the inhibition of orotic acid metabolism by 6-azauridine; neither guanosine nor uridine, at concentrations up to 5mM, relieved the 84% inhibition by 6-azauridine (7.5mM) of $^{14}CO_2$ generation from carboxy-labeled ^{14}C-orotic acid (3). In the experiments with rat mammary gland, 6-azauridine was not included in the reaction mixtures.

While both guanosine and uridine inhibited the incorporation of $NaH^{14}CO_3$ into orotic acid, neither nucleoside was observed to affect the rate of incorporation of ^{14}C-carbamoylphosphate (^{14}C-CP) or ureido-^{14}C-carbamoylaspartate (^{14}C-CA) in either of the tissues examined (TABLE I). Thus the purine nucleoside or its metabolites appear to inhibit the de novo biosynthesis of pyrimidines in both the chick oviduct and the rat mammary gland at the same site as does uridine, that is, at the reaction catalyzed by carbamoylphosphate synthetase.

The relative sensitivity of the orotate pathway to inhibition by purine and pyrimidine nucleosides is illustrated for each tissue in Figures 1 and 2. While the incorporation of $NaH^{14}CO_3$ into orotic acid in the chick oviduct is equally sensitive to inhibition by guanosine and uridine (Fig 1), the purine nucleosides inhibit the de novo biosynthesis of orotic acid in mammary gland to a much greater extent (more than 90%) than does uridine (about 50%), with adenosine being more effective than guanosine at the lower concentrations (Fig 2). The differences in sensitivity to adenosine and guanosine in mammary gland may reflect differences in permeability, endogenous pool sizes, and the rate of metabolism of the two purine nucleosides, as well as differences in their inhibitory activity.

It has been reported recently that phosphoribosylpyrophosphate (PRPP) serves as an activator of the glutamine-dependent carbamoylphosphate synthetase (4) which raises the possibility that the purine nucleosides are inhibiting the incorporation of $NaH^{14}CO_3$ into orotic acid in our studies by depleting the cell of PRPP in the conversion of free purine bases, generated from the added nucleosides, to the mononucleotides via the purine "salvage" pathway. However, two lines of evidence argue against this interpretation: (1) depletion of PRPP would also inhibit the conversion of orotic acid to orotidine monophosphate and cause an accumulation of ^{14}C-labeled orotate; in fact, much less ^{14}C-orotate is isolated in the presence of purines when $NaH^{14}CO_3$ is employed as a precursor and no change in the amount of ^{14}C-orotate iso-

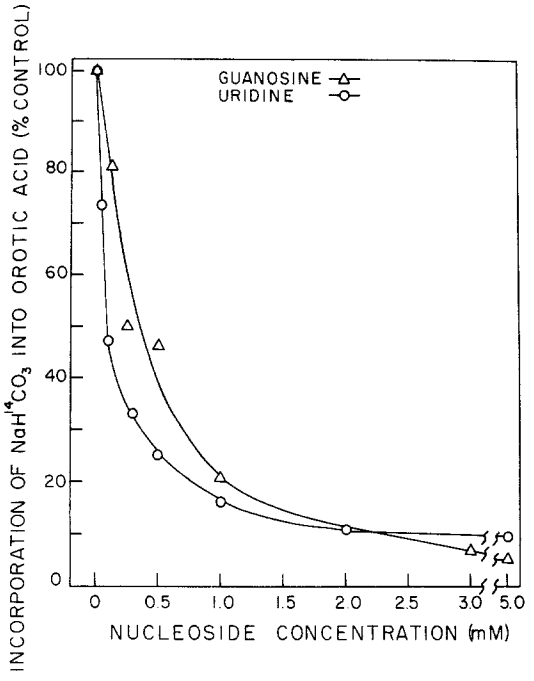

FIGURE 1. THE EFFECT OF GUANOSINE AND URIDINE ON THE INCORPORATION OF $NaH^{14}CO_3$ INTO OROTIC ACID IN MINCES OF CHICK OVIDUCT-The experimental conditions were the same as those described in TABLE I except that guanosine or uridine was added at the concentrations indicated. The uninhibited activities were 27 and 40 nanomoles $NaH^{14}CO_3$ incorporated into orotic acid/gm oviduct · hr for the slope obtained with guanosine and uridine, respectively.

lated is detected when ^{14}C-CP or ^{14}C-CA are employed as precursors (TABLE I), and (2) in an experiment comparing the inhibitory activity of nucleosides and their corresponding nucleotides, the incorporation of $NaH^{14}CO_3$ into orotic acid was inhibited 80% by adenosine, AMP, or ATP and 58-68% by guanosine, GMP, or GTP, each employed at a concentration of 1mM; that these low levels of nucleotides would be degraded to the free bases in sufficient quantity to deplete the cells of PRPP through salvage activity seems unlikely.

These data provide evidence that purine nucleosides, or their metabolites, inhibit the de novo biosynthesis of pyrimidines in the intact cell by interaction with the glutamine-dependent carbamoylphosphate synthetase. The above observations offer an explanation of the results in an earlier study by Krooth in which adenosine was found to retard cell growth in culture and

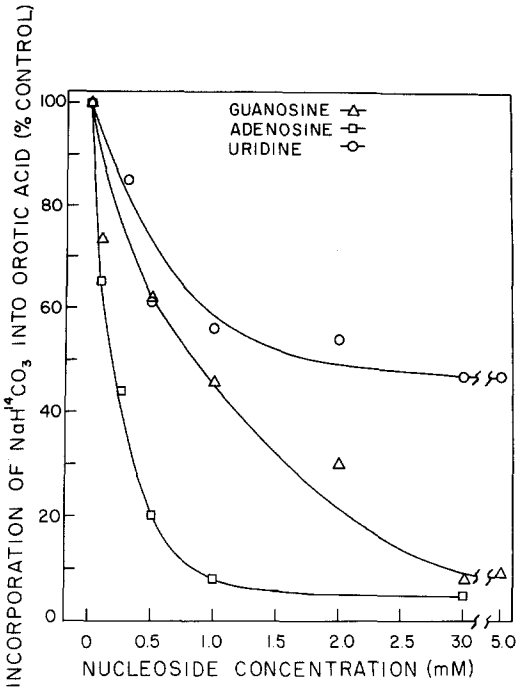

FIGURE 2. THE EFFECT OF PURINE AND PYRIMIDINE NUCLEOSIDES ON THE INCORPORATION OF $NaH^{14}CO_3$ INTO OROTIC ACID IN MINCES OF RAT MAMMARY GLAND-The experimental conditions were the same as those described in TABLE I except that purine or pyrimidine nucleosides were added at the concentrations indicated. The average value obtained in these experiments in 8 determinations of the control activity was 19 ± 2.3 (S.E.) nanomoles of $NaH^{14}CO_3$ incorporated into orotic acid/gm tissue · hr.

lower the level of the enzymes catalyzing the conversion of orotic acid to UMP, presumably by depleting the cell of intermediates of the orotate pathway which induce these enzymes (5). Inhibition of the glutamine dependent carbamoylphosphate synthetase by purines might also explain the more recent observation that adenosine induces pyrimidine starvation and cell death in cultured fibroblasts and lymphoid cells (6, 7).

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