CONTROL OF PYRIMIDINE BIOSYNTHESIS IN RAT LIVER BY FEEDBACK INHIBITION OF ASPARTATE CARBAMOYLTRANSFERASE*

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SUMMARY: Studies on the incorporation of labeled precursors into orotic acid in slices of rat liver reveal aspartate carbamoyltransferase to be a site for the feedback control of hepatic pyrimidine biosynthesis through end-product inhibition.

While conducting studies on the source of carbamoylphosphate for the biosynthesis of hepatic pyrimidines (1, 2) we found that the incorporation of labeled bicarbonate into orotic acid by tissue slices is inhibited by the addition of uridine to the incubation medium (Fig. 1). The feedback inhibition reaches a maximum of 87% at 5mM uridine and is observed with concentrations of uridine as low as 0.5mM. The low concentrations at which uridine is effective in reducing the incorporation of bicarbonate into orotic acid testify to the physiological significance of this means of regulation of the orotate pathway in rat liver.

We proceeded to localize the site of feedback inhibition by comparing the effect of uridine on the incorporation of various precursors into orotic acid. The addition of uridine to the incubation medium was found to inhibit the incorporation of bicarbonate and carbamoylphosphate, but not carbamoylaspartate; feedback control of pyrimidine biosynthesis in rat liver appears to occur at the reaction catalyzed by aspartate carbamoyltransferase and possibly that catalyzed by carbamoylphosphate synthetase as well (Table I.).

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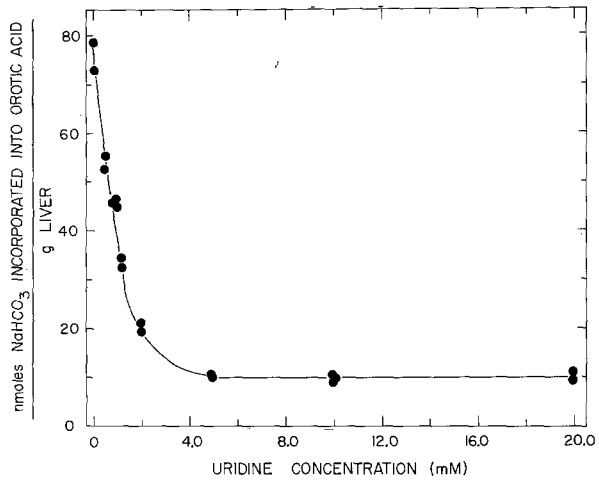

Figure 1. THE EFFECT OF URIDINE ON THE INCORPORATION OF BICARBONATE INTO OROTIC ACID - The experimental conditions were the same as those described earlier (1) except that 6-azauridine (10mM) was added routinely to inhibit the conversion of orotic acid to UMP. Similar results are obtained in the absence of 6-azauridine but the further metabolism of orotic acid under such conditions reduces the accuracy of measurements of the rate of incorporation of precursor into orotic acid (2). The reaction was terminated by the addition of an equal volume of ice-cold 1N $HClO_4$. The acidified reaction mixture was homogenized, the particulate matter removed by centrifugation, and the supernatant fluid neutralized with KOH; the resultant precipitate of $KClO_4$ was removed by centrifugation. ^{14}C-labeled orotate was isolated from the neutralized supernatant fluid by co-crystallization with unlabeled monosodium orotate with which the supernatant fluid was saturated at 90-100° C. The resultant crystals were re-crystallized to constant specific activity from which data the rate of incorporation of bicarbonate into orotic acid was calculated; treatment of the supernatant fluid with a preparation of OMP pyrophosphorylase and OMP decarboxylase prior to saturation with unlabeled monosodium orotate resulted in the isolation of crystals containing no detectable radioactivity, thereby confirming the identity of the co-crystallized radioactive material as ^{14}C-labeled orotate (2).

While the addition of uridine had no effect on the incorporation of carbamoylaspartate into orotic acid at either low (1mM) or high (5mM) concentrations of carbamoylaspartate, it was shown to inhibit the incorporation of carbamoylphosphate most effectively at the lower concentration of carbamoylphosphate tested (81% inhibition at 1mM vs 55% inhibition at 5mM) suggesting that the inhibition produced by the addition of uridine could be overcome by increasing the concentration of carbamoylphosphate. This interpretation was confirmed

Table I. THE EFFECT OF URIDINE ON THE INCORPORATION OF LABELED PRECURSORS INTO OROTIC ACID IN TISSUE SLICES OF RAT LIVER- Experimental conditions were the same as those described in the legend in Figure 1, except that ^{14}C-carbamoylphosphate (^{14}C-CP) and ^{14}C-carbamoylaspartate (^{14}C-CA) were substituted for ^{14}C-$NaHCO_3$ where indicated.

Precursor	Uridine Concentration (mM)	Rate of incorporation (nmoles/g liver · hr)	% Control
^{14}C-$NaHCO_3$, 4.7mM	0 (Control)	78.5	100
^{14}C-$NaHCO_3$, 4.7mM	5	10.1	13
^{14}C-CP, 5mM	0 (Control)	775	100
^{14}C-CP, 5mM	5	350	45
^{14}C-CP, 1mM	0 (Control)	103	100
^{14}C-CP, 1mM	5	20	19
^{14}C-CA, 5mM	0 (Control)	62.5	100
^{14}C-CA, 5mM	5	57.6	92
^{14}C-CA, 1mM	0 (Control)	8.2	100
^{14}C-CA, 1mM	5	8.2	100

by examining the influence of the concentration of carbamoylphosphate on its rate of incorporation into orotic acid in the presence and absence of uridine. A plot of reaction velocity against substrate concentration produces a sigmoidal curve which suggests that aspartate carbamoyltransferase of rat liver possesses more than one catalytic site, the binding of substrate with one catalytic site enhancing the further binding of substrate with any additional catalytic sites (Fig. 2). The addition of uridine to the reaction mixture shifts the curve to the right and exaggerates its sigmoidal shape. Uridine appears to act as a competitive inhibitor of carbamoylphosphate since the addition of uridine to the reaction mixture increases the requirement for carbamoylphosphate needed to reach half-maximum velocity but does

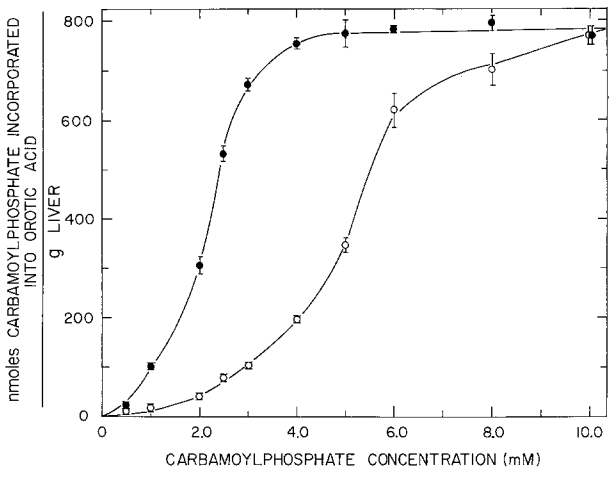

Figure 2. PREVENTION OF THE END-PRODUCT INHIBITION OF ASPARTATE CARBAMOYLTRANSFERASE BY CARBAMOYLPHOSPHATE- Experimental conditions were the same as those described in the legend to Figure 1 except that ^{14}C-carbamoylphosphate was used, at the concentrations indicated, as the labeled precursor. The incorporation of ^{14}C-carbamoylphosphate into orotic acid was determined with (—o—) and without (—•—) added uridine (5mM). Each value represents the average of three separate experiments ± the standard deviation.

not alter the maximum velocity; the inhibition by uridine is prevented by increasing the concentration of carbamoylphosphate. These kinetics show a striking similarity to those reported by Gerhart and Pardee for aspartate carbamoyltransferase of Escherichia coli except that end-product inhibition could be prevented by high concentrations of aspartate and not carbamoylphosphate (3) whereas end-product inhibition of aspartate carbamoyltransferase in rat liver slices is prevented by high concentrations of carbamoylphosphate (Fig. 2) and not aspartate (2). Although the chemical identity of the feedback inhibitor remains to be established, it seems likely that the inhibitor is a metabolite of uridine rather than uridine itself since inhibition of the incorporation of carbamoylphosphate into orotic acid is also observed with the addition of the nucleotides of either uridine or cytidine (2).

While numerous attempts have been made to gain evidence for the feedback control of mammalian aspartate carbamoyltransferase by end-product inhibition, the results thus far have been largely negative (cf. 4-8). The characteristics of aspartate carbamoyltransferase from rat liver reported in this communication were not observed with

fractionated homogenates or with the partially purified enzyme prepared from the same source (5, 8, 9). Apparently, disruption of the cell leads to an alteration of the enzyme which renders it insensitive to both the action of substrate as a positive effector and feedback control by end-product inhibition since both properties are absent in the partially purified preparations (5, 8) but clearly present when the enzyme activity is observed in tissue slices.

Although we find the rate of incorporation of bicarbonate into orotic acid in tissue slices of brain, kidney, and spleen to approach that found in liver slices, the addition of uridine to the reaction mixtures containing these extrahepatic tissues is without effect; thus, it appears from these studies that there is no feedback control of pyrimidine biosynthesis in brain, kidney, or spleen at either the level of aspartate carbamoyltransferase or the cytoplasmic carbamoylphosphate synthetase (2). The possibility that the mitochondrial carbamoylphosphate synthetase of rat liver is also under feedback control remains to be studied but seems remote since the kidney also possesses this enzyme and the incorporation of bicarbonate into orotic acid in kidney slices is insensitive to the addition of uridine.

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