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RESPONSES OF HEPATIC PHOSPHO*ENOL*PYRUVATE CARBOXYKINASE ACTIVITIES FROM NORMAL AND DIABETIC RATS TO QUINOLINATE INHIBITION AND FERROUS ION ACTIVATION *

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Summary

1. Phospho*enol*pyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32) from tryptophan-treated normal rats, when assayed immediately after preparation is not activated by Fe^{2+} but is inhibited 65% by 2.0 mM quinolinate whether or not Fe^{2+} is present. As time of storage increases, the enzyme's sensitivity to Fe^{2+} activation returns as does the ability of quinolinate to more effectively inhibit the Fe^{2+}-activated enzyme.

2. Phospho*enol*pyruvate carboxykinase from NaCl- and tryptophan-treated diabetic rats is activated about 2-fold by 20 μM Fe^{2+}. Quinolinate (2.0 mM) inhibits the Fe^{2+}-activated enzyme 65% compared to 20% inhibition of the non-Fe^{2+}-activated enzyme. In these respects, the enzyme from NaCl- and tryptophan-treated diabetic rats acts in vitro just like the enzyme from NaCl-treated normal rats and unlike the enzyme from tryptophan-treated normal rats. Thus, the inability of tryptophan and quinolinate to inhibit gluconeogenesis and to alter the assayable activity of phospho*enol*pyruvate carboxykinase from diabetic rats in vivo is inconsistent with quinolinate's ability to inhibit the enzyme in vitro.

3. Quinolinate's inhibition of phospho*enol*pyruvate carboxykinase from

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NaCl, tryptophan-treated normal and diabetic rats is of a 'mixed' nature.

4. Hepatic cytosolic phospho*enol*pyruvate carboxykinases from fasted normal guinea pigs, pigeons, and rabbits are activated 2—3-fold by Fe^{2+} and inhibition by quinolinate in the presence of Fe^{2+} ranges from 65—75% compared to no inhibition without Fe^{2+}. Mitochondrial carboxykinases from these three species are only activated 20—30% by Fe^{2+}, although quinolinate, which is ineffective as an inhibitor in the absence of Fe^{2+}, inhibits the enzymes 40—50% in the presence of Fe^{2+}.

Introduction

Alloxan-induced diabetes in rats is characterized by high levels of blood glucose and is relatively unresponsive to feeding or starvation. Normal regulatory controls appear to be altered or absent and hepatic gluconeogenesis proceeds at an accelerated rate [1].

Phospho*enol*pyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32) is considered to be a major site of regulation of gluconeogenesis [2]. Tryptophan, or more precisely its metabolite quinolinate, inhibits gluconeogenesis in normal rats by inhibiting phospho*enol*-pyruvate carboxykinase but concomitantly causes a paradoxical increase in the assayable activity of the enzyme [3—6].

A number of in vitro studies have focused on the ability of Fe^{2+} to inactivate phospho*enol*pyruvate carboxykinase from normal rats and on quinolinate's ability to inhibit the Fe^{2+}-activated enzyme [5—7]. Conversely, the elevated activity of the enzyme from tryptophan-treated normal rats is equally sensitive to inhibition by quinolinate whether or not Fe^{2+} is present [7]. Recently a cytosolic protein called ferroactivator has been implicated as being necessary for the Fe^{2+} activation of cytosolic phospho*enol*pyruvate carboxykinase [8—10] and the quantity of ferroactivator has been found to increase under conditions such as starvation or diabetes, which are conducive to gluconeogenesis [11].

Tryptophan given to intact diabetic rats or their isolated livers exerts no inhibitory effects on the conversion of lactate to glucose, nor does it alter the in vitro activity of phospho*enol*pyruvate carboxykinase [12]. But the kynurenine pathway of tryptophan metabolism is reportedly aberrant in diabetic rats, thereby causing decreased production of quinolinate [13—16]. However, quinolinate is also ineffective in inhibiting gluconeogenesis or enhancing the in vitro activity of phospho*enol*pyruvate carboxykinase in diabetic rats [12]. This insensitivity of carboxykinase activity from diabetic rats to tryptophan or quinolinate may be due to changes in the properties of the enzyme. To test this hypothesis we have compared the in vitro responses of phospho*enol*pyruvate carboxykinases from NaCl- and tryptophan-treated normal and diabetic rats to Fe^{2+} and to quinolinate and these results are reported here. Also reported are in vitro responses to Fe^{2+} and/or quinolinate of both mitochondrial and cytosolic preparations of phospho*enol*pyruvate carboxykinases from several other species. A preliminary report of these data has been made before the American Society of Biological Chemists [17].

Materials and Methods

Animals. Male Sprague-Dawley rats (180—200 g) from ARS-Sprague-Dawley (Madison, WI, U.S.A.) were given Purina Rat chow and tap water ad libitum. Male New Zealand rabbits (approx. 1 kg) and male guinea pigs (approx. 200 g) from Gopher State Caviary (St. Paul, MN, U.S.A.), were provided with Purina Rabbit and Guinea Pig chow respectively, along with tap water ad libitum. Male pigeons from Mogul-Ed Co. (Oshgosh, WI, U.S.A.) were given cracked corn and tap water. Feeding procedures were altered as required by experimental protocol.

L-Tryptophan treatment of intact rats. L-Tryptophan was suspended in 0.9% NaCl and injected intraperitoneally at a dose of 75 mg/kg body wt. Controls were injected with an equivalent dose of NaCl.

Induction of alloxan diabetes. 48 h fasted rats were injected via the tail vein with recrystallized alloxan monohydrate (40 mg/kg body wt.) in 0.9% NaCl. Rats were immediately refed and provided with 1% glucose in tap water for 48 h which was then replaced with tap water. Rats were used 7—10 days later if their blood sugar levels were at least 400 mg/100 ml.

Mitochondrial and cytosolic preparations from rat, guinea pig, pigeon and rabbit livers. All animals were decapitated, bled and their livers quickly removed and placed in ice-cold 0.25 M sucrose. Livers were subsequently homogenized in ice-cold 0.25 M sucrose, diluted to volumes (in ml) 10-fold their weight (in g) and centrifuged at $600 \times g$ for 10 min at 2°C to sediment unbroken cells, nuclei and debris. The supernatant fractions were removed, the pellets resuspended, washed once in 0.25 M sucrose and centrifuged as before. The combined supernatant fractions were centrifuged at $15\,000 \times g$ for 10 min to sediment mitochondria which were ultimately washed and reprecipitated twice. The combined supernatant fractions were centrifuged at $105\,000 \times g$ for 1 h. The supernatant fractions were decanted from the pellets and divided into aliquots for immediate assay or frozen to be used later. Mitochondria were diluted to volumes 4 times the liver weights, frozen and thawed 3 times (to liberate mitochondrial phospho*enol*pyruvate carboxykinase) and again immediately assayed or frozen for future use.

Assay of cytosolic and mitochondrial phosphoenolpyruvate carboxykinase. Enzyme preparations were initially assayed on the day of preparation and prior to freezing. Preparations were subsequently stored at −10°C until needed, at which time aliquots were freshly thawed, used and discarded. Phospho*enol*-pyruvate carboxykinase was assayed according to Nordlie and Lardy [18] as modified by Foster et al. [5,6,19]. The reaction mixture normally contained 1.6 μmol GSH/6.7 μmol oxalacetate/9.0 μmol Na_3HITP/22.5 μmol $MgCl_2$/20 μmol KF/94 μmol Tris-HCl and up to 0.2 ml of enzyme in a final volume of 1.5 ml, pH 8.0. Reactions were initiated by addition of enzyme to pre-incubated reaction mixture and, after 5 min at 30°C were normally terminated with 6% $HClO_4$. For kinetic experiments, reactions were terminated with KBH_4 [20] and the phospho*enol*pyruvate measured [21]. Protein was measured by the biuret method [22] with bovine serum albumin as a standard. The specific activity of phospho*enol*pyruvate carboxykinase is expressed in nmol phospho-*enol*pyruvate formed per min per mg protein.

Fe^{2+} activation experiments. When studying Fe^{2+} activation of phospho*enol*pyruvate carboxykinase, enzyme-containing solutions were pre-incubated in 20 μM $FeCl_2$ for 7 min at 0°C at which time 0.2 ml of enzyme · Fe^{2+}-containing preparation was added to 1.3 ml of reaction mixture. We used 20 μM Fe^{2+} rather than the 30 μM Fe^{2+} used by Snoke et al. [7] as several unpublished experiments indicated no significant differences in the effects of the two concentrations of metal.

Quinolinate inhibition studies. Quinolinic acid was usually added to reaction mixtures to a final concentration of 2.0 mM except for the kinetic experiments in which case 0.25 mM quinolinate was used.

Materials. L-Tryptophan was from Mann Research Laboratories (New York, NY, U.S.A.); $FeCl_2$ was from Mallinckrodt Chemical Works (St. Louis, MO, U.S.A.); Na_3HITP, NADH and ATP were from P-L Biochemicals Inc. (Milwaukee, WI, U.S.A.); pyruvate kinase, lactate dehydrogenase and oxaloacetate were from Boehringer Mannheim Corp. (New York, NY, U.S.A.) and quinolinic acid was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Chemicals were of the highest grade available and used as received except that quinolinic acid was recrystallized from 7 M acetic acid and dried in vacuo over NaOH pellets prior to use. All solutions were prepared in deionized glass-distilled water.

Results

Response of phosphoenolpyruvate carboxykinase from NaCl- or tryptophan-treated normal and diabetic rats to Fe^{2+} and/or quinolinate

Data presented in Fig. 1 show that the activity of phospho*enol*pyruvate carboxykinase from tryptophan-treated normal rats when assayed immediately after preparation is approx. 2—3-fold above normal (spc. act. about 80) and is inhibited about 65% by 2.0 mM quinolinate whether or not Fe^{2+} is present. This confirms the observations reported by Snoke et al. [7]. It is also

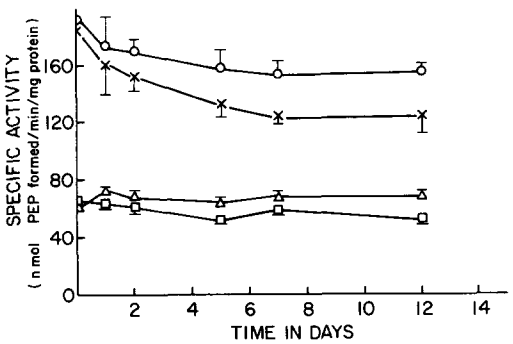

Fig. 1. The effect of time of storage at −10°C on Fe^{2+} activation and quinolinate inhibition of hepatic cytosolic phospho*enol*pyruvate carboxykinase from tryptophan-treated normal rats. The specific activity of the enzyme is plotted as a function of time. Normal rats were fasted for 24 h and treated for 4 h with vehicle or with 75 mg tryptophan/100 g body wt. The enzyme activity was assayed alone, ×; after 7 min preincubation at 0°C with 20 μM $FeCl_2$, ○; in the presence of 20 mM quinolinate, △; and in the presence of 2.0 mM quinolinate after 7 min pre-incubation with 20 μM $FeCl_2$, □. Each point represents the mean results ±1 S.D. from experiments with three rats. PEP, phospho*enol*pyruvate.

TABLE I

THE INFLUENCE OF QUINOLINATE ON THE KINETIC PARAMETERS OF PHOSPHO*ENOL*PYRUVATE CARBOXYKINASE PREPARATIONS FROM NaCl- AND TRYPTOPHAN-TREATED, 24 h FASTED, NORMAL AND DIABETIC RATS

Kinetic studies were performed with oxalacetate concentrations ranging from 0.094 to 0.313 mM in the reaction mixture and under conditions defined in Materials and Methods. The concentration of Fe^{2+} used during pre-incubation was 20 μM; the concentration of quinolinate used was 0.25 mM. Values for K_m oxalacetate and V for Phospho*enol*pyruvate formation and their standard deviations were calculated by use of computer-fitted regression lines for the reciprocal data and the comparison of data obtained in the presence of quinolinate vs · those obtained in its absence were tested via use of the Student's t-test. Data analyzed were from a minimum of three experiments performed with pooled enzyme preparations from three rats. $-Fe^{2+}$ — quinolinate K_m oxalacetate (μM) and $-Fe^{2+}$ — quinolinate; V, phospho*enol*pyruvate formation (pM).

Source of preparation	$-Fe^{2+}$				$+Fe^{2+}$			
	—quinolinate		+quinolinate		—quinolinate		+ quinolinate	
	K_m	V	K_m	V	K_m	V	K_m	V
Normal + NaCl	80 ± 21	16.5 ± 1.5	244 ± 54 *	12.4 ± 1.6 **	40 ± 16	26.1 ± 2.2	240 ± 83 *	11.7 ± 2.3 **
Diabetic + NaCl	72 ± 9	37.2 ± 1.4	275 ± 82 *	29.5 ± 3.6 **	108 ± 23	64.2 ± 5.8	441 ± 120 *	29.7 ± 5.7 **
Normal + tryptophan	60 ± 18	23.6 ± 1.9	183 ± 80 *	18.5 ± 4.1 **	48 ± 20	26.1 ± 2.6	218 ± 18 *	12.5 ± 4.4 **
Diabetic + tryptophan	89 ± 21	38.0 ± 3.2	290 ± 90 *	36.8 ± 7.6	92 ± 22	62.0 ± 5.4	880 ± 510 *	50.7 ± 23.6 ***

* K_m in presence of quinolinate significantly different from K_m in its absence at $p < 0.001$.

** V in presence of quinolinate significantly different from V in its absence at $p < 0.001$.

*** V in presence of quinolinate significantly different from V in its absence at $p < 0.05$.

apparent from Fig. 1 that the activity of the enzyme from tryptophan-treated normal rats decays with time of storage as initially reported by Foster et al. (Ref. 6, Fig. 2). Furthermore, we find that the enzyme from tryptophan-treated rats when assayed immediately after its preparation, is not activated by Fe^{2+}. However, as time of storage at −10°C increases and activity decays, the enzyme's sensitivity to Fe^{2+} returns, as does the ability of quinolinate to preferentially inhibit the Fe^{2+}-activated enzyme. So that after 12 days of storage, Fe^{2+} activates the enzyme by 25% and quinolinate inhibits the Fe^{2+}-activated enzyme by 68%, compared to 46% inhibition of the non-activated enzyme.

Based on the above observations, phospho*enol*pyruvate carboxykinase preparations from NaCl- and tryptophan-treated diabetic rats were examined in terms of their stabilities to storage and their responses to Fe^{2+} and/or quinolinate. Although data were not presented, the activities of enzyme preparations from both NaCl- and tryptophan-treated diabetic rats were predictably elevated due to their diabetic condition [5,19]. However, both enzyme preparations were also found to be stable to storage, activated approx. 2-fold by Fe^{2+}, and more sensitive to inhibition by quinolinate in the presence of Fe^{2+} (approx. 65%) as opposed to its absence (15—20%). All of these observations are exactly analogous to those made with carboxykinase preparations from NaCl-treated normal rats.

To study the mechanism of action of quinolinate on phospho*enol*pyruvate carboxykinase, a series of kinetic experiments were performed on the nature of quinolinate's in vitro inhibition of the enzyme's activity in the absence and presence of Fe^{2+}. Table I shows data from such experiments performed with enzyme preparations from NaCl- and tryptophan-treated normal and diabetic rats in the absence and presence of Fe^{2+}.

It is apparent that quinolinate in both the absence and presence of Fe^{2+} significantly increases the apparent K_m oxalacetate of enzyme preparations from all four groups of rats, normal and diabetic, tryptophan-treated or not. Quinolinate also significantly decreases the V of all enzyme preparations except that from diabetic rats treated with tryptophan and assayed in the absence of Fe^{2+}. These data collectively indicate that quinolinate's inhibition of phospho*enol*pyruvate carboxykinase is of a mixed type.

The effect of quinolinate and/or Fe^{2+} on the activities of cytosolic and mitochondrial phosphoenolpyruvate carboxykinases from livers of different species

Because of quinolinate's ability to partially inhibit gluconeogenesis in isolated livers and/or hepatocytes from fasted guinea pigs [23] and rabbits [24] but not pigeons [23], we investigated the in vitro effect of Fe^{2+} and quinolinate alone and together on mitochondrial and cytosolic activities of phospho*enol*pyruvate carboxykinase from these species. The cellular distribution of the enzyme's activity is approx. 3/10 and 1/1 cytosol/mitochondria in fed and fasted quinea pigs, respectively; approx. 1/3 and 3/2 cytosol/mitochondria in fed and fasted rabbits, respectively and approx. 1/8 cytosol/mitochondria in both fed and fasted pigeons [25].

Data presented in Table II indicate that cytosolic phospho*enol*pyruvate carboxykinases from pigeons, guinea pigs and rabbits are all activated 2—3-fold

TABLE II

THE INFLUENCE OF QUINOLINATE AND/OR Fe^{2+} ON HEPATIC MITOCHONDRIAL AND CYTOSOLIC PHOSPHO*ENOL*-PYRUVATE CARBOXYKINASE ACTIVITIES FROM VARIOUS SPECIES

Specific activities expressed as nmol phospho*enol*pyruvate formed per min per mg protein. Number of animals given in parenthesis.

Species	Specific activity of phospho*enol*pyruvate carboxykinase			
	Control	20 μM Fe^{2+}	2.0 mM Quinolinate	Fe^{2+} + Quinolinate
Cytosol				
Pigeon (6)	42.9 ± 16.7 *	95.5 ± 31.0	39.3 ± 14.4	34.4 ± 10.8
Guinea pig (7)	29.6 ± 8.4	73.2 ± 17.3	27.8 ± 7.0	18.1 ± 5.6
Rabbit (4)	21.0 ± 7.9	65.7 ± 23.0	18.9 ± 4.2	17.6 ± 6.3
Mitochondria				
Pigeon (6)	348 ± 34	420 ± 54	326 ± 31	238 ± 23
Guinea pig (7)	91.1 ± 7.7	120 ± 12	84.9 ± 5.1	59.1 ± 6.2
Rabbit (4)	120 ± 18	147 ± 21	113 ± 17	66.8 ± 4.8

* Mean results ± 1 S.D.

by Fe^{2+} and that quinolinate, which has no inhibitory effect in the absence of Fe^{2+}, inhibits the activities of the Fe^{2+}-activated enzymes 65—75%.

Other data in Table II indicate that the Fe^{2+} activates mitochondrial carboxykinase from these three species only 20—30%. Nonetheless, quinolinate which is essentially ineffective as an inhibitor in the absence of Fe^{2+}, significantly inhibits the Fe^{2+}-activated enzymes 40—50%.

Discussion

L-Tryptophan given to normal rats inhibits gluconeogenesis by inhibition of phospho*enol*pyruvate carboxykinase [3,5] although quinolinic acid is actually responsible for the inhibition [4]. Such inhibition of carboxykinase in intact rats and isolated livers by tryptophan and quinolinate, respectively, is accompanied by a paradoxical increase in the enzyme's assayable activity, which decays rapidly during storage toward the level of activity in normal control rats [5,6]. Foster et al. [6] interpret the decay in activity as implying that the form of enzyme from tryptophan-treated normal rats reverts to the form of stable enzyme found in normal control rats.

Snoke et al. [7] find that phospho*enol*pyruvate carboxykinase from NaCl-treated normal rats is activated about 2-fold by Fe^{2+} and the Fe^{2+}-activated enzyme is inhibited about 60% by quinolinate. Conversely, we find that the enzyme from tryptophan-treated normal rats is not activated by Fe^{2+} but is, even in the absence of Fe^{2+}, inhibited 65% by quinolinate as reported by Snoke. We also find that the decay of carboxykinase activity from tryptophan-treated normal rats is accompanied by both a return of sensitivity to Fe^{2+} activation and a greater inhibition of the Fe^{2+}-activated enzyme by quinolinate, such responses being analogous to those made with enzyme from normal control rats. Thus our findings are consistent with the above-mentioned interpretation, that the form of carboxykinase from tryptophan-treated normal rats reverts to the form found in normal controls.

Alvares and Ray [12] find that tryptophan given to intact alloxan-diabetic rats and quinolinate given to isolated diabetic livers neither inhibits gluconeogenesis nor alters the assayable activity of phospho*enol*pyruvate carboxykinase and these findings have been confirmed [26,27]. Nonetheless, we find that the enzyme from both NaCl- and tryptophan-treated diabetic rats is activated approx. 2-fold by Fe^{2+} and Fe^{2+}-activation is required for quinolinate to exert significant inhibitory effects. Furthermore, tryptophan-treatment of diabetic rats does not cause instability of the enzyme's activity to storage. Since phospho*enol*pyruvate carboxykinase from both NaCl- and tryptophan-treated diabetic rats responds in vitro to Fe^{2+} and/or quinolinate just as does the enzyme from NaCl-treated normal rats, some possibilities are presented which might explain the in vivo observations of Alvares and Ray [12].

For example, the nature of the phospho*enol*pyruvate carboxykinase molecule and/or its intracellular environment as (the content and redox state of various metal ions) in diabetic rats could be modified such that, the enzyme's activity is unresponsive to tryptophan given to intact diabetic rats or to quinolinate given to isolated diabetic livers. However, we have shown that the enzyme from NaCl-treated diabetic rats responds predictably to Fe^{2+} and/or quinolinate in vitro.

The lack of influence of tryptophan or 3-hydroxyanthranilate influence on gluconeogenesis or on the assayable activity of phospho*enol*pyruvate carboxykinase of diabetic rats [12], could be due simply to a diabetes-induced decrease in their absorption and/or metabolism to quinolinate in quantities sufficient to exert the usual effects. However, this possibility is presumably negated by the fact that quinolinate given to isolated diabetic livers is also ineffective in inhibiting gluconeogenesis or increasing the assayable activity of the enzyme [12]. It seems unlikely that diabetes would alter hepatic membranes to the extent that quinolinate would not be absorbed.

Evidence is accumulating which supports the involvement of ferroactivator in the activation of phospho*enol*pyruvate carboxykinase by Fe^{2+} and a physiological role of this protein in regulation of gluconeogenesis [8—11]. However, the mechanism by which ferroactivator exerts its influence is presently unknown, thus making it impossible to speculate on the relationship of ferroactivator to the observations described herein. It does seem certain that a deficiency of ferroactivator is not a causative factor since its hepatic content is increased by starvation and alloxan-diabetes [11].

McDaniel et al. [28] report that 1 mM quinolinic acid inhibits phospho*enol*pyruvate carboxykinase (in the absence of Fe^{2+}) in a non-competitive manner whereas 2 mM quinolinic acid causes a mixed type of inhibition. The same authors, working with enzyme presented simultaneously with both Fe^{2+} and quinolinic acid, report competitive inhibition at limiting concentrations of oxalacetate. Our data presented in Table I shows quinolinate's inhibition of carboxykinase to be of a 'mixed' type and suggest little competition between oxalacetate and 0.25 mM quinolinate, whether or not the enyzme is activated by pre-incubation with added Fe^{2+}. In other words, we find that quinolinate alters both the apparent V and K_m for oxalacetate of phospho*enol*pyruvate carboxykinase. Such differences in these findings may be of considerable interest in interpreting data related to the actual nature and location of interac-

tion between quinolinate and the enzyme molecule.

Can tryptophan or quinolinate inhibit gluconeogenesis in species other than rats? Tryptophan inhibits glucose formation from lactate and alanine by fasted mouse hepatocytes [29] and quinolinate partially inhibits alanine-derived gluconeogenesis in isolated fasted guinea pig livers [23], but is ineffective as an inhibitor of gluconeogenesis in isolated fasted pigeon livers [23]. Based on differential effects of amino-oxyacetate and quinolinate on gluconeogenesis in isolated pigeon, rat and guinea pig livers, Söling, Kleineke and co-workers [23,25] concluded that quinolinate does not cross the mitochondrial membrane and thereby only inhibits cytosolic phospho*enol*pyruvate carboxykinase. Based on studies of quinolinate's uptake by isolated rat and rabbit livers, they further concluded that quinolinate does not cross the plasma membrane into the cells of rabbit liver, thereby explaining their findings and those of Huibregtse et al. [30] that quinolinate does not inhibit gluconeogenesis in isolated rabbit livers. Conversely, Zaleski and Bryla [24] reported that quinolinate causes an approximate 50% inhibition of lactate-derived gluconeogenesis in fasted rabbit hepatocytes. Fürst et al. [31] found that administration of tryptophan to humans given a mixture of amino acids after three days on a carbohydrate-free diet, decreases glucose and urea production and produces a metabolic profile in arterial and hepatic vein blood indicative of inhibition of hepatic phospho*enol*pyruvate carboxykinase. Apparently, tryptophan or quinolinate is capable of inhibiting gluconeogenesis, at least partially, in every species tested (except pigeons, where hepatic carboxykinase is nearly completely localized in mitochondria).

Given that tryptophan or quinolinate inhibits gluconeogenesis by inhibition of cytosolic but not mitochondrial phospho*enol*pyruvate carboxykinase, what are the in vitro influences of Fe^{2+} and/or quinolinate on the activities of mitochondrial and cytosolic carboxykinase from various species other than rats? Data presented in Table II indicate that the cytosolic activities of phospho*enol*-pyruvate carboxykinase from pigeons, guinea pigs and rabbits are all activated 2—3-fold by Fe^{2+} and only after Fe^{2+} activation does quinolinate cause significant inhibition of the cytosolic enzyme from the three species. Conversely, the mitochondrial activities of carboxykinase from pigeons, guinea pigs and rabbits are essentially unresponsive to Fe^{2+} although quinolinate which is ineffective as an inhibitor in the absence of Fe^{2+} is considerably inhibitory in the presence of Fe^{2+}. These observations are in accord with those of MacDonald [11,32] who also finds Fe^{2+} to be ineffective in activating mitochondrial carboxykinase activities from a variety of species, although the presence of Fe^{2+} greatly enhances the inhibitory effects of 3-mercaptopicolinate. Thus, it appears that the presence of Fe^{2+}, even though it does not cause acitvation, is nonetheless necessary for inhibition of mitochondrial phospho*enol*pyruvate carboxykinase by either inhibitor and our data, like those of MacDonald, support the presence of a divalent metal binding site in mitochondrial as well as cytosolic phospho-*enol*pyruvate carboxykinase. Our data also suggest that the inability of quinolinate to inhibit mitochondrial production of phospho*enol*pyruvate in vivo could be related to a lack of availability of Fe^{2+} rather than simply and solely to an inability of quinolinate to cross the mitochondrial membrane. However, the quantity, nature and oxidation state of mitochondrial Fe are currently unknown.

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