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Regulation of Glutamate Dehydrogenase by Mg^{2+} and Magnification of Leucine Activation by Mg^{2+}

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SUMMARY

In the presence of Mg^{2+}, pure glutamate dehydrogenase is more reactive with NADPH than with NADH and is markedly activated by elevations in the ADP/ATP ratio or the addition of leucine. Because these are properties of glutamate dehydrogenase in mitochondria but not properties of the pure enzyme studied in the absence of Mg^{2+}, Mg^{2+} could be a ligand that confers upon glutamate dehydrogenase the regulatory properties of this enzyme found *in situ*. In the absence of the allosteric activators ADP, leucine, or succinyl-CoA, Mg^{2+} is an inhibitor and increases product inhibition by α-ketoglutarate in the forward reaction and substrate inhibition by α-ketoglutarate in the reverse reaction. However, the allosteric activators convert Mg^{2+} from an inhibitor into an activator of the forward reaction. In the reverse reaction, ADP also converts Mg^{2+} from an inhibitor into an activator and leucine eliminates inhibition by Mg^{2+}. Because Mg^{2+} is an inhibitor in the absence of activator that also increases inhibition by α-ketoglutarate, whereas in the presence of activator Mg^{2+} has no effect or is itself an activator, Mg^{2+} magnifies the effect of the activator, and magnification increases with increases in the concentration of α-ketoglutarate. Leucine and its analog 2-aminobicyclo (2.2.1) heptane 2-carboxylic acid (BCH) have almost identical effects on both human and bovine glutamate dehydrogenase in both the presence and absence of Mg^{2+}. However, advantages of BCH over leucine as a potential pharmacological activator of glutamate dehydrogenase are that BCH is not metabolized and, unlike leucine, BCH does not inhibit ornithine transcarbamylase. Isoleucine and valine alone have little effect on human glutamate dehydrogenase, but isoleucine slightly inhibits the enzyme in the presence of leucine.

There is increasing awareness of the potential pharmacological significance of the glutamate dehydrogenase [L-glutamate:NAD(P) oxidoreductase (deaminating) (EC 1.4.1.3)] reaction:

$$\text{Glutamate} + \text{NAD(NADP)} \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH(NADPH)}$$

Branched chain amino acids are of therapeutic value in treating olivopontocerebellar atrophy, amyotrophic lateral sclerosis (1, 2), and NH_4^+ toxicity resulting from liver disease (3), and this could be related to the ability of branched chain amino acids to activate glutamate dehydrogenase. In pancreatic islets, activation of glutamate dehydrogenase by leucine or its nonmetabolized analog BCH is associated with insulin release (4–6), and leucine also stimulates insulin release *in vivo* (7). In addition, we have recently demonstrated that methyl esters of succinate (but not fumarate or malate) are quite insulinotropic (8, 9). The effect of succinate on insulin release could also be due to activation of glutamate dehydrogenase. Succinate, as a substrate for GTP-succinate thiokinase, should both decrease the concentration of GTP and increase the concentration of succinyl-CoA. Consequently, GTP, a potent inhibitor of glutamate dehydrogenase, would be replaced with succinyl-CoA, which we have found to be an activator of glutamate dehydrogenase (9). All rapidly dividing tumors utilize glutamine instead of glucose as a main metabolic fuel (10), in part because in these tumors citrate, unlike glutamate, cannot serve as a precursor for α-ketoglutarate in the α-ketoglutarate dehydrogenase reaction (11). Consequently, pharmacological intervention into unique aspects of glutamate metabolism in tumors might be a way to inhibit tumor growth. This could be related to the ability of glutamate to decrease the oncolytic action of vinblastine (12).

The physiological and potential pharmacological importance of glutamate dehydrogenase justifies a thorough study of the regulation of this enzyme. Therefore, in this paper we have studied the effect of Mg^{2+} on activation of bovine and human glutamate dehydrogenase by leucine, its analog BCH, and other allosteric activators. BCH might be a useful pharmacological agent because, unlike leucine, it is not metabolized and does not activate islet glutaminase (7). Furthermore, leucine inhibits ornithine transcarbamylase (EC 2.1.3.3) (13), whereas the effects of BCH on ornithine transcarbamylase have not been studied previously.

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ABBREVIATION: BCH, 2-aminobicyclo(2.2.1)heptane-2-carboxylic acid.

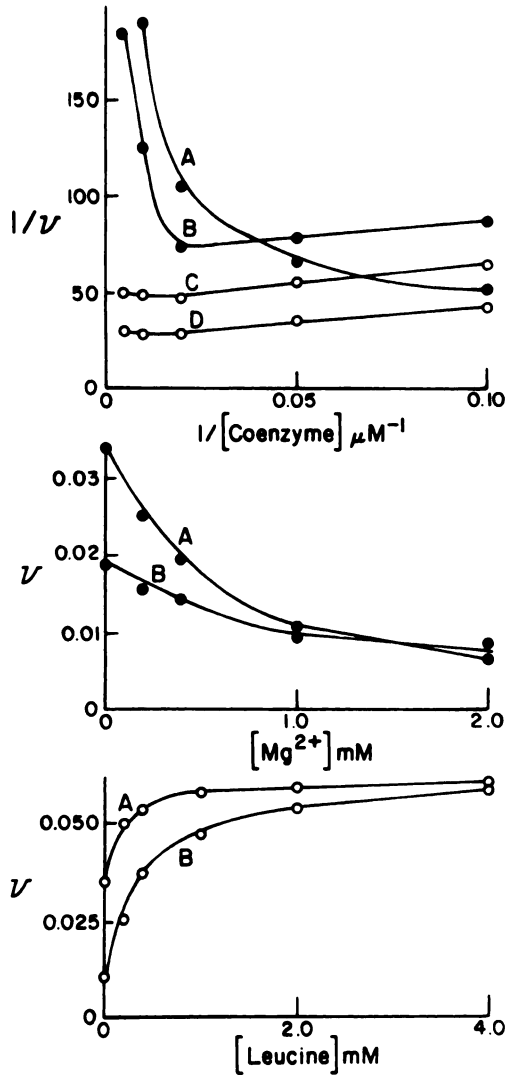

Fig. 1. Double-reciprocal plots of velocity versus reduced coenzyme (*upper*), $MgCl_2$ (*middle*), or leucine (*lower*) concentration. *Upper*, experimental conditions were 2 mM α-ketoglutarate, 50 mM NH_4Cl, plus NADH with 2 mM $MgCl_2$ (*curve A*), NADPH with 2 mM $MgCl_2$ (*curve B*), NADPH (*curve C*), or NADH (*curve D*). *Middle*, experimental conditions were 2 mM α-ketoglutarate, 50 mM NH_4Cl, plus 100 μM NADH (*curve A*) or 100 μM NADPH (*curve B*). *Solid curves*, calculated with Eq. 2, using values of V_i equal zero and K_i equal 0.6 mM (NADH) or 1.2 mM (NADPH). *Lower*, experimental conditions were 100 μM NADH, 50 mM NH_4Cl, 2 mM α-ketoglutarate, plus no additions (*curve A*) or 1 mM $MgCl_2$ (*curve B*). *Solid curves*, calculated with Eq. 1, using values of V_A/V equal to 1.8 (no Mg^{2+}) or 6.3 (plus Mg^{2+}) and K_A equal to 0.15 mM (no Mg^{2+}) or 0.35 mM (plus Mg^{2+}). Additional assay conditions were 20 mM potassium phosphate, 0.1 mM EDTA, 20 μg/ml cytosolic alanine aminotransferase, and 0.17 μg/ml bovine glutamate dehydrogenase, pH 7.0 at 25°. Velocity is in units of change in absorbance at 340 nm/min.

Materials and Methods

Enzymes and reagents. Bovine liver glutamate dehydrogenase and rat liver ornithine transcarbamylase were prepared as described previously (13, 14). Substrates, coenzymes, and other reagents were obtained from Sigma Chemical Company. Stock solutions of all reagents were adjusted to the pH of the assays and prepared as sodium salts. Solutions of succinyl-CoA and pyridine nucleotide coenzyme were prepared fresh daily. The concentration of succinyl-CoA was determined by measuring its absorbance at 260 nm and correcting for the amount of CoA by measuring the concentration of sulhydryl groups, as described previously (15).

Concentrations of enzymes. The concentrations of pure ornithine transcarbamylase and glutamate dehydrogenase were determined as described previously (13, 14). The concentration of protein in human liver mitochondrial extracts was measured with the method of Lowry *et al.* (16).

Enzyme assays. Glutamate dehydrogenase and ornithine transcarbamylase were assayed as described previously (13, 14). Cytosolic alanine aminotransferase (20 μg/ml) was added to assays of glutamate dehydrogenase to stabilize glutamate dehydrogenase, as described previously (9).

Data collection and analysis. The effects of allosteric modifiers of glutamate dehydrogenase in the presence of constant levels of substrates can be expressed by Eq. 1 for an activator (A) and Eq. 2 for an inhibitor (I) (17, 18).

$$\Delta v = \frac{[A](V_A - V)}{[A] + K_A} \quad (1)$$

$$\Delta v = \frac{[I](V - V_i)}{[I] + K_i} \quad (2)$$

In Eqs. 1 and 2, Δv is the change in velocity produced by the modifier, V is the velocity in the absence of modifier, V_A and V_i are the velocities in the presence of saturating modifier, and K_A and K_i are the apparent dissociation constants of the modifier. Because Eqs. 1 and 2 are empirically identical to the Michaelis-Menten equation, data corresponding to Eqs. 1 and 2 were fitted to these equations using the least squares method, as described previously (19). Each experimental point was the average of three experiments. Standard errors were less than 10% of the mean and, therefore, were not shown.

Other procedures. Methods of preparing mitochondrial extracts and measuring enzyme turbidity have been described previously (20, 21).

Results

Inhibition by Mg^{2+}. Reduced coenzyme binds to both an active and an inhibitory site on glutamate dehydrogenase (22). Because the affinity of the active site is higher, K_m and V_{max} can be obtained via linear extrapolation through double-reciprocal plots of velocity versus reduced coenzyme concentration at low concentrations of coenzyme. As shown in Fig. 1 (*upper*, *curve B* versus *curve C*), Mg^{2+} decreased V_{max} and K_m for both NADH and NADPH and markedly increased substrate inhibition (association of reduced coenzyme with the inhibitory site). Substrate inhibition was greater with NADH, so that in the presence of Mg^{2+} and high concentrations of reduced coenzyme the velocity was greater with NADPH than with NADH. The K_i of Mg^{2+} was 0.6 mM in the presence of 100 μM NADH versus 1.2 mM in the presence of 100 μM NADPH (Fig. 1, *middle*), indicating that binding of NADH to the inhibitory site in turn enhanced the affinity of the enzyme for Mg^{2+}.

Binding of reduced coenzyme to the inhibitory site enhances binding of α-ketoglutarate to the oxidized coenzyme-enzyme complex, which results in substrate inhibition by α-ketoglutarate (23, 24). Therefore, Mg^{2+}, by enhancing association of reduced coenzyme with the inhibitory site, also enhanced substrate inhibition by α-ketoglutarate (Fig. 2, *right*, *curve A* versus *curve B*).

In general, allosteric inhibitors of glutamate dehydrogenase increase the value of k_1, the rate of association of reduced coenzyme with the active site (25). Because both the K_m and K_D (Michaelis and dissociation constant, respectively, of reduced coenzyme at the active site) are inversely proportional to k_1, Mg^{2+}, which decreases the K_m, also apparently decreases

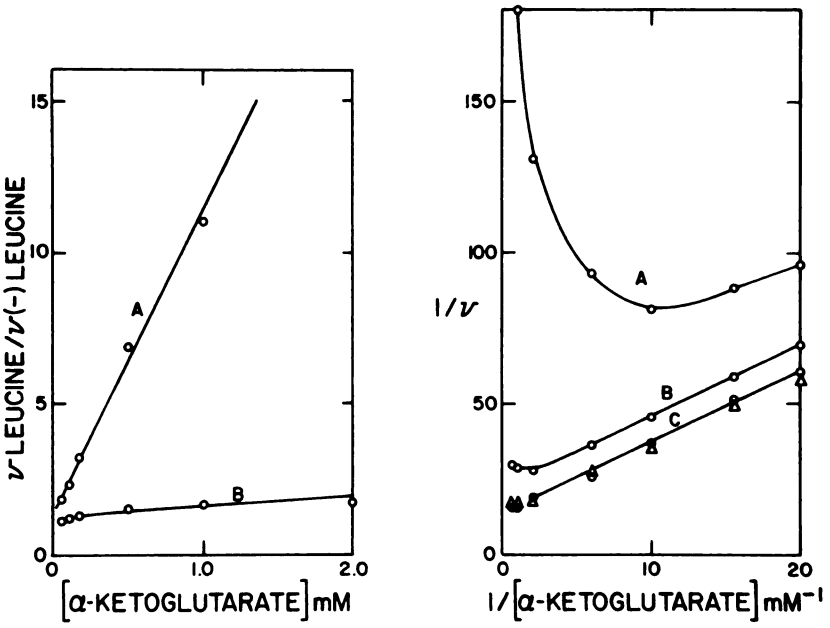

Fig. 2. Plots of the ratio of velocity in the presence to the velocity in the absence of leucine versus α-ketoglutarate concentration (*left*) and double-reciprocal plots of velocity versus α-ketoglutarate concentration (*right*). Assays contained 100 μM NADH, 50 mM NH_4Cl, and the indicated concentration of α-ketoglutarate, plus 2 mM $MgCl_2$ (*curve A*, *right* and *left*), no further additions (*curve B*, *right* and *left*), or 4 mM leucine with or without 2 mM $MgCl_2$ (*curve C*, *right*). Other assay conditions are given in the legend to Fig. 1. Velocity is in units of change in absorbance at 340 nm/min.

TABLE 1

Effect of Mg^{2+} and α-ketoglutarate on the forward glutamate dehydrogenase reaction and magnification of the effect of leucine

The values of the kinetic constants (V_A, V, V_i, and K_i) were calculated with Eqs. 1 and 2. V (the rate in the absence of activator or inhibitor) was given a value of 1.0, and the other velocities were normalized in proportion to this value. V_i' and V_{iA} are the velocities in the presence of the indicated concentration of inhibitor alone or together with a saturating concentration of activator, respectively. The value of V_i (rate in the presence of saturating inhibitor) was zero when either Mg^{2+} or α-ketoglutarate was the inhibitor. The actual experimental data are shown in Fig. 3. Assays were performed in the presence of 1.0 mM NADP and 5.0 mM glutamate. Other experimental conditions are described in the legend to Fig. 3.

Additions			Kinetic constants			
Constant inhibitor	Added activator	Varied inhibitor	V_i'	K_i	V_{iA}	V_A
				mM		
None	None	Mg^{2+}		3.0		
α-Ketoglutarate, 0.2 mM	None	Mg^{2+}		1.8		
None	None	α-Ketoglutarate		0.24		
Mg^{2+}, 1 mM	None	α-Ketoglutarate		0.14		
None	Leucine	α-Ketoglutarate		0.24		
Mg^{2+}, 1 mM	Leucine	α-Ketoglutarate		0.32		
None	Leucine	None				1.8
Mg^{2+}, 2 mM	Leucine	None	0.65		3.6	
α-Ketoglutarate, 0.2 mM	Leucine	None	0.65		1.7	
α-Ketoglutarate, 0.2 mM, plus Mg^{2+}, 2 mM	Leucine	None	0.27		4.5	

K_D or decreases dissociation of reduced coenzyme from the active site (25). Several kinetic consequences can result from Mg^{2+} decreasing K_D. First, dissociation of reduced coenzyme from the active site is the rate-limiting step of the forward reaction (26). Consequently, Mg^{2+} also inhibited the forward reaction (Table 1 and Fig. 3, *upper*, *curve C*). Second, the reverse glutamate dehydrogenase reaction is ordered (24, 27) so that binding of reduced coenzyme to the active site is required for significant binding of α-ketoglutarate to the active complex. Consequently, Mg^{2+}, by enhancing binding of reduced coenzyme to the active site, also decreased the K_m of α-ketoglutarate in the reverse reaction (Fig. 2, *right*, *curve A* versus *curve B*). Finally, α-ketoglutarate can inhibit the forward reaction by associating with the reduced coenzyme-enzyme complex, even in the presence of levels of glutamate that are sufficiently high to prevent binding of α-ketoglutarate to the oxidized coenzyme-enzyme complex (8, 24). Consequently, Mg^{2+}, by enhancing the stability of the reduced coenzyme-enzyme complex, decreased the K_i of α-ketoglutarate in the forward reaction, even in the presence of levels of glutamate 10-fold greater than the K_m of glutamate (Fig. 3, *lower*, *curve C* versus *curve D*, Table 1, and Ref. 6). Enhanced binding of α-ketoglutarate to the active complex in turn decreased the K_i of Mg^{2+} in the forward reaction (Fig. 3, *upper*, *curve C* versus *curve D*, and Table 1).

Under these assay conditions (20 mM potassium phosphate, 0.1 mM EDTA, pH 7.0), only 0.3 to 0.4 mM Mg^{2+} would be free when the total level of Mg^{2+} is 1 to 2 mM (28, 29). The remainder would be associated with PO_4^{2-}, EDTA, and to a lesser extent substrates. A level of free Mg^{2+} of 0.3 to 0.4 mM is in the range of the buffered level of free Mg^{2+} (0.3 mM) found in normal mitochondria (30). Therefore, because the K_i of Mg^{2+} (in terms of total Mg^{2+} concentration) was lower than 2 mM, Mg^{2+} would be a significant regulator of glutamate dehydrogenase in mitochondria. Furthermore, the significance of Mg^{2+} as a regulator would increase as the concentration of α-ketoglutarate and reduced coenzyme is increased.

Mg^{2+}-activator interaction. According to the above results, Mg^{2+} enhances binding of reduced coenzyme to both

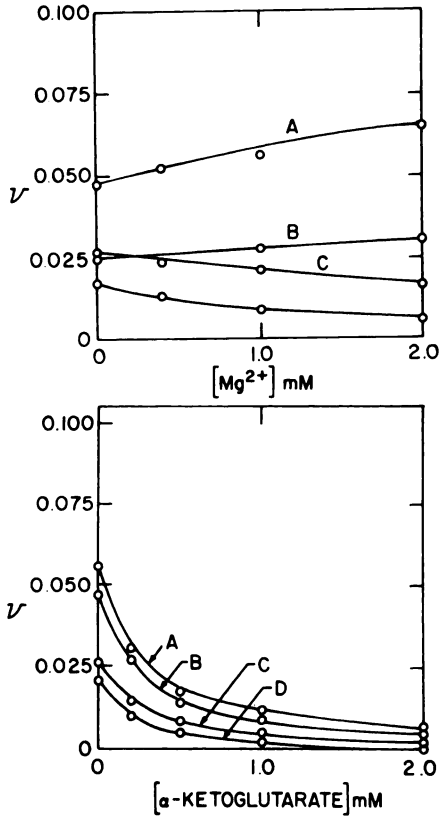

Fig. 3. Plot of velocity versus Mg^{2+} (*upper*) or α-ketoglutarate (*lower*) concentration. Experimental conditions were 1.0 mM NADP, 5.0 mM glutamate, and 0.5 μg/ml bovine glutamate dehydrogenase. Remaining experimental conditions are described in the legend to Fig. 1. *Upper*, additions were 4 mM leucine (*curve A*), 4 mM leucine plus 0.2 mM α-ketoglutarate (*curve B*), none (*curve C*), or 0.2 mM α-ketoglutarate (*curve D*). *Lower*, additions were 2 mM leucine plus 1 mM $MgCl_2$ (*curve A*), 2 mM leucine (*curve B*), none (*curve C*), or 1 mM $MgCl_2$ (*curve D*). *Solid curves*, calculated with Eqs. 1 or 2. *Upper*, values of the constants were $V_A/V = 1.8$ and $K_A = 2.0$ mM (*curve A*) and $V_i = 0$ (*curves C* and *D*) and $K_i = 3.0$ mM (*curve C*) and 1.8 mM (*curve D*). *Lower*, values of the constants were V_i equal to zero for all four curves and K_i equal to 0.32, 0.24, 0.24, and 0.14 mM in *curves A*, *B*, *C*, and *D*, respectively. Velocity is in units of change in absorbance at 340 nm/min.

active and inhibitory sites, which enhances binding of α-ketoglutarate to complexes between the enzyme and reduced and oxidized coenzyme, respectively. These effects are opposite those of the allosteric activator leucine (23, 26). Leucine increases the K_m of reduced coenzyme and α-ketoglutarate, decreases substrate inhibition by reduced coenzyme and α-ketoglutarate, and activates both the forward and reverse reaction (23, 26). Because leucine and Mg^{2+} have opposite effects, high concentrations of leucine eliminated the effect of Mg^{2+} on the reverse reaction (Fig. 1, *lower*, *curve A versus curve B*, and Fig. 2, *right*, *curve C*). This was not due to leucine competitively displacing Mg^{2+} from the enzyme because, in the presence of NADPH, Mg^{2+} enhanced enzyme turbidity even in the presence of saturating concentrations of leucine (Fig. 4). Furthermore, in the forward reaction, leucine was clearly not competitive with Mg^{2+} because it, like the activators ADP and succinyl-CoA, converted Mg^{2+} from an inhibitor into an activator (Fig. 3, *upper*, *curve A* versus *curve C*, and Table 2). Therefore, Mg^{2+} is bound to the activator-enzyme complex, and in the forward reaction this facilitates the ability of the activator to increase enzyme activity by enhancing dissociation of reduced coenzyme

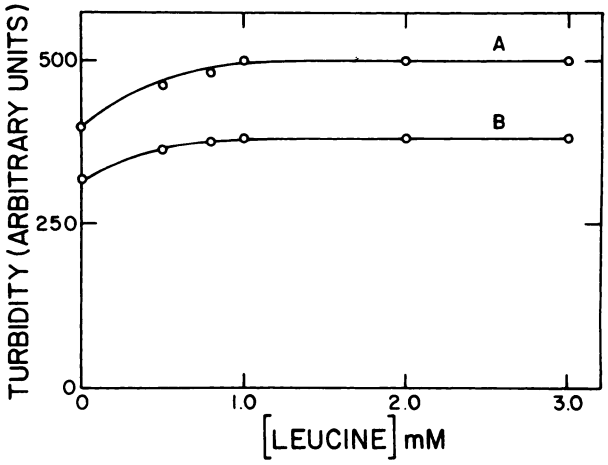

Fig. 4. Plot of enzyme turbidity versus concentration of leucine. Additions were 2 mM $MgCl_2$ (*curve A*) and none (*curve B*). These experiments were performed with 1.0 mg/ml bovine glutamate dehydrogenase in the presence of 100 μM NADPH, 20 mM potassium phosphate, and 0.1 mM EDTA, pH 7.0 at 25°.

TABLE 2

Magnification of effect of activators of the forward glutamate dehydrogenase reaction by Mg^{2+}

The values of the kinetic constants (V, V_A, and K_A) were determined with Eq. 1. V (the rate in the absence of activator and $MgCl_2$) was given a value of 1.0 and the other velocities were normalized to this value, so that V_i' (the rate in the presence of 2 mM $MgCl_2$) equaled 0.69. V_M is the normalized velocity in the presence of 2 mM $MgCl_2$ and saturating levels of activator. Assays were performed in the presence of 1.0 mM NADP and 5.0 mM glutamate. Other experimental conditions are described in the legend to Fig. 1.

Activator	K_A		Kinetic constants			
	$-Mg^{2+}$	$+Mg^{2+}$	V_A	V_M	V_A/V	V_M/V_i'
	μM					
Leucine	140	200	1.8	2.5	1.8	3.6
Succinyl-CoA	40	100	1.3	2.4	1.3	3.5
ADP	3.1	8.4	1.8	2.6	1.8	3.7

TABLE 3

Magnification of activation of reverse glutamate dehydrogenase reaction by Mg^{2+}

The values of the kinetic constants were determined with Eq. 1 and normalized as described in the legend to Table 2. In the reverse reaction, the normalized value of V_i' (rate in presence of 2 mM $MgCl_2$) was 0.37. Assays were performed in the presence of 100 μM NADPH, 50 mM NH_4Cl, and 2.0 mM α-ketoglutarate. Other experimental conditions are described in the legend to Fig. 1.

Added activator	K_A		Kinetic constants			
	$-Mg^{2+}$	$+Mg^{2+}$	V_A	V_M	V_A/V	V_M/V_i'
	μM					
Leucine	570	1900	1.8	1.8	1.8	4.9
ADP	9.5	80	1.3	5.1	2.3	13.8

from the active site. ADP and leucine are not potent chelators (26, 29). Therefore, the synergism between these activators and Mg^{2+} in the forward reaction does not result from binding of a Mg-activator complex to the enzyme. In contrast, when ATP, a potent chelator, was the activator, we as others (29) found that Mg^{2+} decreased activation, because Mg-ATP has a low affinity for the enzyme.

Because the combination of Mg^{2+} plus activator facilitated dissociation of reduced coenzyme from the active site, this combination also decreased binding of α-ketoglutarate to the reduced coenzyme-enzyme complex. For example, in the exper-

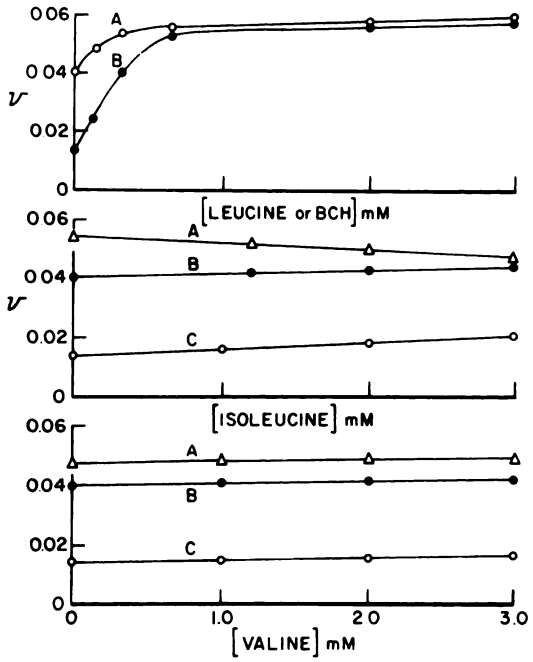

Fig. 5. Plot of human liver glutamate dehydrogenase activity versus concentration of amino acid activator. *Upper*, the concentration of either leucine or BCH was varied in either the presence (*curve B*) or absence (*curve A*) of 2 mM $MgCl_2$. *Middle*, the level of isoleucine was varied in the presence of 0.4 mM leucine (*curve A*), no additions (*curve B*), or 2 mM $MgCl_2$ (*curve C*). *Lower*, results obtained when the concentration of valine was varied in the presence of 0.2 mM leucine (*curve A*), no addition (*curve B*), or 2 mM $MgCl_2$ (*curve C*). These experiments were performed with human liver mitochondrial extract in the presence of 100 μM NADH, 50 mM NH_4Cl, and 2 mM α-ketoglutarate. Remaining conditions are given in the legend to Fig. 1.

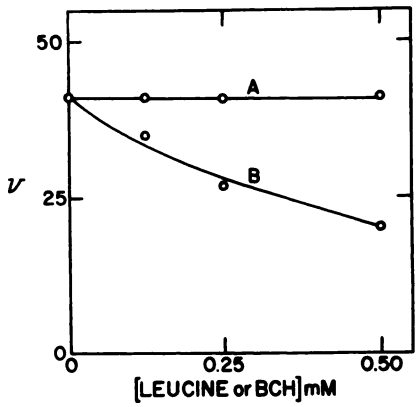

Fig. 6. Plot of the velocity of ornithine transcarbamylase versus the concentration of leucine or BCH. These assays were performed with rat liver ornithine transcarbamylase plus the indicated concentrations of either BCH (*curve A*) or leucine (*curve B*), as described previously (13). Experimental conditions were 5.0 mM ornithine, 2.5 mM carbamyl phosphate, 50 mM triethanolamine, pH 7.8 at 25°. Velocity is in units of nmol of citrulline produced/ml/10-min incubation.

iment summarized in Table 1, the K_i for α-ketoglutarate in the presence of 1 mM $MgCl_2$ was increased from 0.14 to 0.32 mM by leucine (see also Fig. 3, *lower*, *curve A* versus *curve D*).

In the absence of Mg^{2+}, the K_i of α-ketoglutarate was the same (0.24 mM) in the forward reaction in either the presence or absence of leucine (Table 1 and Fig. 3, *curve B* versus *curve C*). Leucine apparently does not alter the K_i in the absence of Mg^{2+}, because leucine increases the K_m of glutamate (8). Consequently, although leucine decreases association of α-ketoglutarate with the reduced coenzyme-enzyme complex, association of α-ketoglutarate with the oxidized coenzyme-enzyme complex is enhanced, so that these opposing actions apparently cancel. Saturating levels of glutamate are required for leucine to eliminate product inhibition by α-ketoglutarate (8).

Magnification of the effects of activator by Mg^{2+}. According to Eq. 1, the magnitude of the effect of saturation levels of an activator equals V_A/V. In the presence of a constant level of inhibitor, the magnitude of the effect of saturating activator can be expressed as V_{iA}/V_i' where V_{iA} equals the velocity in the presence of inhibitor and saturating activator and V_i' equals the velocity in the presence of inhibitor alone. Mg^{2+} inhibited glutamate dehydrogenase in the absence of leucine (V_i' less than V), but in the presence of leucine Mg^{2+} either had no effect (V_{iA} equaled V_A, reverse reaction) or was itself an activator (V_{iA} exceeded V_A, forward reaction). Therefore, V_{iA}/V_i' exceeds V_A/V (Tables 1–3) or Mg^{2+} magnified the effect of leucine. Furthermore, because the inhibitory effect of Mg^{2+} in the absence of leucine increased as the concentration of α-ketoglutarate was increased but Mg^{2+} had an opposite effect in the presence of leucine, magnification by Mg^{2+} increased as the concentration of α-ketoglutarate was increased (Fig. 1, *left*, and Table 1). In the reverse reaction, Mg^{2+} produced even greater magnification for ADP than for leucine, because ADP converted Mg^{2+} from an inhibitor into an activator of the reverse reaction (Table 3).

Human glutamate dehydrogenase. We have previously demonstrated that the effects of allosteric inhibitors and activators of glutamate dehydrogenase are quite similar with both the human and bovine liver glutamate dehydrogenase (8). This was also true for Mg^{2+}. In the absence of leucine it inhibited both enzymes completely, whereas in the presence of saturating leucine it activated the forward reaction (not shown) and had no effect on the reverse reaction (Fig. 5, *upper*). Consequently, Mg^{2+} also magnified activation of the human enzyme by leucine.

Isoleucine and valine alone had little effect on the human enzyme with or without Mg^{2+}, but isoleucine slightly inhibited the enzyme in the presence of leucine (Fig. 5, *middle*, *curve A*).

Effect of BCH. BCH and leucine had almost identical effects on both the bovine and human liver enzymes (Fig. 5, *upper*). However, BCH, unlike leucine, was not an inhibitor of ornithine transcarbamylase (Fig. 6).

Discussion

According to the results presented in this paper, Mg^{2+} decreases the difference between the regulatory behavior of pure glutamate dehydrogenase and glutamate dehydrogenase in mitochondria. In mitochondria, glutamate dehydrogenase is more reactive in the reverse reaction with NADPH than with NADH (31), is activated as much as 8-fold by leucine (23, 32, 33), and is activated by an increase in the ADP/ATP ratio (34, 35). These are properties of pure glutamate dehydrogenase in the presence of Mg^{2+}. Therefore, Mg^{2+} could be a ligand that confers upon glutamate dehydrogenase the regulatory properties of this enzyme found in mitochondria. Because Mg^{2+} produces a greater enhancement of substrate inhibition by NADH than by NADPH, glutamate dehydrogenase can be more reactive in the presence of Mg^{2+} with NADPH than with NADH. ADP and ATP are almost equivalent activators of the pure enzyme in the absence of Mg^{2+}. However, Mg^{2+} decreases activation by

ATP, because Mg-ATP has a low affinity for the enzyme (29). Alternatively, Mg^{2+} enhances activation by ADP, and ADP converts Mg^{2+} from an inhibitor into an activator. Mg^{2+} increases activation by leucine from 2-fold to over 6-fold, because in the absence of leucine it is an inhibitor that increases inhibition by α-ketoglutarate, whereas in the presence of leucine it has no effect (reverse reaction) or is itself an activator (forward reaction).

Other ligands can also magnify the effects of the activators leucine, ADP, and succinyl-CoA on glutamate dehydrogenase and, thus, make this enzyme more susceptible to pharmacological activation. The allosteric inhibitors GTP, palmitoyl-CoA, and Zn^{2+} magnify the effects of activators, because saturating levels of activator completely restore the velocity to the level found in the presence of activator and absence of inhibitor (9, 17, 21–23, 25, 26, 36–38). In the case of GTP and palmitoyl-CoA, the allosteric activators competitively displace the inhibitor (9, 17, 22, 23, 25, 26, 36–38) and, although Zn^{2+} is not bound to the enzyme in the same region as activators, the activator can increase the dissociation constant of Zn^{2+} so that it dissociates from the enzyme (21). Furthermore, the effect of leucine on inhibition by Zn^{2+} is magnified by Mg^{2+}, which facilitates displacement of Zn^{2+} by leucine (21).

The interactions described above could take place in mitochondria. The level of free Mg^{2+} required for modification of glutamate dehydrogenase is within the range of free Mg^{2+} found in mitochondria (30). However, in diabetes, where the level of free Mg^{2+} is low (30), Mg^{2+} might be a less potent regulator of glutamate dehydrogenase. α-Ketoglutarate is a known inhibitor of glutamate dehydrogenase in mitochondria (35). The mitochondrial level of NADH is above the level required for substrate inhibition in the presence of Mg^{2+} (39, 40). Palmitoyl-CoA [K_i, 0.03 μM (36)] has a higher affinity for glutamate dehydrogenase than for any other protein (41). The K_i of Zn^{2+} [0.5 μM (42)] could be lower than the mitochondrial level of Zn^{2+}, especially in pancreatic islets where the mitochondrial level of Zn^{2+} is quite high (43), and Zn^{2+} is apparently an endogenous inhibitor of glutamate dehydrogenase (44). Therefore, it is probable that the combined action of these inhibitors plus Mg^{2+} would magnify the pharmacological effects of leucine, esters of succinate, and BCH on mitochondrial glutamate dehydrogenase. An exception might be GTP, because the Mg-GTP complex has a low affinity for some glutamate dehydrogenases (9, 29).

Presently, combinations of branched chain amino acids are being used pharmacologically in the treatment of olivopontocerebellar atrophy, amyotrophic lateral sclerosis, and hepatic failure (1–3). Part of their efficacy could be secondary to activation of glutamate dehydrogenase. However, valine and isoleucine have little effect on the human enzyme, and isoleucine even slightly inhibits the action of leucine. BCH has not been tested as a pharmacological agent, although it is as effective as leucine in activating human glutamate dehydrogenase in either the presence or absence of Mg^{2+}. Potential advantages of BCH are that it is not metabolized and does not inhibit ornithine transcarbamylase. Although our conclusions are based upon experiments performed with liver enzymes, we and several others have not found marked differences between the regulatory properties of liver and brain glutamate dehydrogenase when both are studied under the same experimental conditions (23, 45, 46).

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