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An Overview of γ-Hydroxybutyrate Catabolism: The Role of the Cytosolic NADP$^+$-Dependent Oxidoreductase EC 1.1.1.19 and of a Mitochondrial Hydroxyacid-Oxoacid Transhydrogenase in the Initial, Rate-Limiting Step in This Pathway

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γ-Hydroxybutyrate (GHB) is a naturally occurring compound present in micromolar concentration in both brain (1,2) and in peripheral tissues (3). This endogenous compound is remarkable in that pharmacological doses of 200–500 mg/kg produce marked behavioral and electroencephalographic changes (4), a profound decrease in cerebral glucose utilization (5), an increase in striatal dopamine levels (6) and a decrease in body temperature (7). High doses of GHB have also been reported to protect neurons (8) and intestinal epithelium (9) against cell death resulting from experimental ischemia. Behavioral changes are not seen with doses of less than 30 mg/kg, but low doses stimulate the release of prolactin, growth hormone and cortisol (10,11), and doses of 5–10 mg/kg result in an increase in body temperature (12). These actions, and the discovery of high affinity binding sites for GHB in the central nervous system (13), suggest that GHB may have a biological function. Both the origin of endogenous GHB and its catabolism are, therefore, of considerable interest.

This review will cover the early work on the degradative pathway for GHB and the discovery of a dual pathway for the initial step in the oxidative catabolism of GHB. The factors which regulate the activity of the enzymes in these pathways, and as a consequence, regulate tissue levels of GHB are also discussed.

Walkenstein et al. (14) established that GHB is largely disposed of, *in vivo* by oxidation to CO_2 and water. These investigators could not find the ^{14}C label from GHB in succinate in urine, but they did find that the label could be trapped in hippuric acid in the urine of animals treated with sodium benzoate as might be expected if GHB were undergoing β-oxidation. They therefore proposed that GHB was metabolized by β-oxidation (14). Möhler et al. (15) and Doherty et al. (16), however, assayed citric acid cycle intermediates in the tissues of animals given [^{14}C]GHB and demonstrated that the carbon skeleton of GHB indeed does enter the citric acid cycle as succinate rather than as acetyl-CoA as would be expected if GHB were being oxidized through the β-oxidation pathway.

They proposed the following pathway:

1) GHB $\rightleftharpoons$ succinic semialdehyde
2) Succinic semialdehyde $\rightarrow$ succinate
3) Succinate $\rightarrow\rightarrow\rightarrow\rightarrow$ Co_2 + H_2O

The discovery of a metabolic disease in which GHB and succinic semialdehyde (SSA) are markedly elevated (17) in both blood and urine due to a block in SSA dehydrogenase (18) added evidence to support a degradative pathway in which GHB is oxidized to SSA, which in turn is oxidized to succinate.

At the time the pathway shown above was proposed, it was known that the enzymes of the citric acid cycle catalyzed the reactions in step (3) and that SSA

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Abbreviations used in this paper: GHB, γ-hydroxybutyrate; SSA, succinic semialdehyde; DTT, dithiothreitol.

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dehydrogenase (the enzyme missing in patients with GHB aciduria (18)) catalyzed the reaction in step (2). Step (1) is always depicted as an essential part of this scheme. However, at the time this pathway was proposed, an enzyme or enzymes which could catalyze this step had not been identified.

Isolation of a Cytosolic GHB Dehydrogenase. Two unusual oxidoreductases, one cytosolic (19) and the other mitochondrial (20), that catalyze the oxidation of GHB to SSA have now been isolated. The cytosolic enzyme, which will be referred to as GHB-dehydrogenase in this review, was first purified to homogeneity from hamster liver (19) and was found to be an $NADP^+$-dependent oxidoreductase. A study of the substrate specificity of purified GHB-dehydrogenase revealed that D-glucuronate and L-gulonate, the product of D-glucuronate reduction, were also good substrates (19). The physical characteristics, as well as the substrate and inhibitor specificity of this enzyme, indicate that the ability to catalyze the oxidation of GHB probably represents a previously unreported activity for the $NADP^+$-dependent oxidoreductase (EC 1.1.1.19) commonly known as D-glucuronte reductase (21). This enzyme may also be identical to the group of enzymes categorized in a 1985 review by Cromlish et al. (22) as "ALR-1", the high K_m aldehyde reductase or L-hexonate dehydrogenase.

Although the oxidation of GHB catalyzed by this GHB-dehydrogenase proceeds at an easily measurable rate when assayed in vitro under optimal conditions, the very low activity found in the in vitro system under conditions simulating those in the cytosol raises the question of how, or indeed whether, this enzyme catalyzes the oxidation of GHB in vivo. An answer to this question may have been found when it was discovered that GHB dehydrogenase could catalyze the reduction of D-glucuronate coupled to the oxidation of GHB (23) as shown below:

$$\text{GHB} + \text{NADP}^+ \rightleftharpoons \text{SSA} + \text{NADPH} + \text{H}^+$$

$$\text{D-glucuronate} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{L-gulonte} + \text{NADP}^+$$

The overall or "coupled" reaction is:

$$\text{GHB} + \text{D-glucuronate} \rightleftharpoons \text{SSA} + \text{L-gulonate}$$

When the kinetic constants for the coupled system were determined, it was found that they were more favorable to oxidation of GHB under conditions present in cytosol of most tissues than were those for the uncoupled system.

The time course of the coupled reaction (Figure 1) in which both products, SSA and NADPH, were measured, shows that in the presence of an adequate concentration of D-glucuronate, only a very small net amount of NADPH is formed even though SSA formation is proceeding rapidly. This is in contrast to the control reaction mixture without glucuronate in which NADPH and SSA are formed in stoichiometric amounts (19). These results indicate that NADPH is being used for the reduction of D-glucuronate at the same rate at which it is being produced by the oxidation of GHB. This would account for the low steady state level of NADPH seen in Figure 1. The effect of increasing concentrations of D-glucuronate on the rate of oxidation of GHB to SSA in the presence of limiting amounts of $NADP^+$ and inhibitory amounts of NADPH is shown in Figure 2. Under these conditions, 2 mM D-glucuronate increased SSA formation 8-fold.

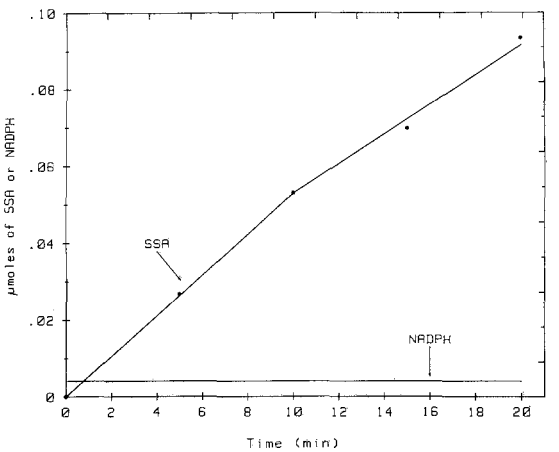

Fig. 1. Time course of succinic semialdehyde (SSA) and NADPH formation in the presence of D-glucuronate (23). The reaction mixture contains 10 mM GHB, 1.0 mM D-glucuronate, 0.1 mM $NADP^+$, 80 mM phosphate buffer, pH 7.6, enzyme and sufficient water to bring the volume to 1.0 ml. SSA and NADPH were determined as previously described (19).

Other important changes in the kinetic constants for this reaction occur in the presence of D-glucuronate (Table I). The K_m (4.5×10^{-4}M) for the coupled reaction is five-fold lower and the V_{max} (1.52 µmol/min/mg protein) is 1.8 times higher than in the uncoupled reaction. The effects of coupling and changes in pH on the rate of degradation of GHB are, however, more accurately described by changes in k (the first order rate constant for the reaction) than they are by changes in V_{max}. The concentration of GHB in the tissues is much lower than K_m and under these conditions the quantity V_{max}/K_m provides a good approximation of k (24). In the coupled reaction, the rate constant for GHB degradation, V_{max}/K_m, is increased 9-fold as compared to the 1.8-fold increase in V_{max} at saturating concentrations of GHB and $NADP^+$ (Table I). The effect of D-glucuronate on the rate of the reaction is much greater at the very low sub-

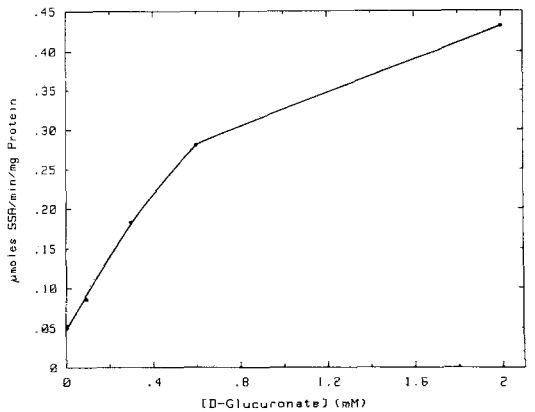

Fig. 2. The effect of D-glucuronate concentration on the rate of conversion of GHB to succinic semialdehyde (SSA) in a reaction mix containing NADPH and a low concentration of NADP+ (23). Reaction mixture: 10 mM GHB, 0.01 mM NADP+, 0.02 mM NADPH, 80 mM phosphate, pH 7.6, enzyme, D-glucuronate as indicated and water to 1 ml. The assay for SSA was carried out as previously described (19).

strate concentrations found *in vivo* than in the standard in vitro assay where V_{max} conditions are used. In the coupled reaction the K_m for NADP+ is decreased from 2×10^{-5} to 1.4×10^{-6}M and the inhibition produced by NADPH ($K_i = 7 \times 10^{-6}$M) has been eliminated (Figure 3). GHB can now be oxidized in the presence of an otherwise extremely inhibitory concentration of NADPH. All of the kinetic constants for the coupled reaction are closer to the tissue concentration range shown in Table I (25) than are those for the uncoupled reaction.

The rate of GHB oxidation is pH-dependent. Earlier work had shown that the pH optimum for the cytosolic oxido-reductase under V_{max} conditions was 9.0 (19), but at a more physiological pH (7.0–7.2) the enzyme was only half as active. The pH optimum was dependent on GHB concentration in both the coupled and uncoupled reactions and in both cases was above 8.0 when saturating concentrations of substrate were used (26). As the concentration of GHB decreases toward levels found in vivo the pH optimum for the coupled reaction shifts toward pH 7.0 (26). A plot of V_{max}/K_m against pH (Figure 4) shows that, at substrate concentrations near those found in vivo, the pH optimum approaches the intracellular pH, i.e. 7.5 for the uncoupled reaction and 7.0 or lower for the coupled reaction (26). Vayer et al. (27) subsequently reported a pH optimum of 8.0 under different conditions from those described above. In their system the oxidation of GHB catalyzed by the cytosolic oxidoreductase was coupled to both the reduction of D-glucuronate and the transamination of SSA to form GABA.

GHB dehydrogenase is inhibited by a number of products of intermediary metabolism (Table II) which includes the ketone bodies, α-ketoglutarate and branched α-ketoacids derived from amino acid degradation as well as degradation products of phenylalanine (26). As has been found with certain aldehyde reductases (28,29), anticonvulsants such as barbiturates, diphenylhydantoin and valproate are good inhibitors of GHB dehydrogenase (30). In addition, GHB dehydrogenase is inhibited by salicylates (30).

GHB dehydrogenase like lysozyme, ribonuclease and a number of other proteins (26), may contain disulfide bridges which are essential for its activity. It is inhibited by compounds such as β-mercaptoethanol and dithiothreitol (DTT), which can reduce disulfide bonds (26). DTT has the most pronounced effect; addition of 2.5 mM DTT produces an 85% inhibition of the activity

Table I. The Effect of D-Glucuronate on the Kinetic Constants for γ-Hydroxybutyrate (GHB), NADP+, and NADPH

		Kinetic Constants		Tissue concentration
		Uncoupled assay	Coupled assay	(range between brain, liver, kidney, muscle)[a]
GHB	$K_{m_{app}}$	2.3×10^{-3} M	4.5×10^{-4} M[b]	$0.1–5 \times 10^{-5}$ M
NADP+	$K_{m_{app}}$	2×10^{-5} M	1.4×10^{-6} M[c]	$2 - 11 \times 10^{-6}$ M
NADPH	K_i	7×10^{-6} M	No inhibition	$1 - 30 \times 10^{-5}$ M
GHB	V_{max}[d]	0.83	1.52[b]	
V_{max}/K_{mGHB}	(k)[e]	0.36	3.38	

[a]The tissue concentrations of GHB are from reference (3). The molar concentration of NADP+ and NADPH in the various tissues were calculated from data taken from reference (25).
[b]1 mM D-glucuronate.
[c]2 mM D-glucuronate.
[d]μmol/min/mg protein.
[e]first order rate constant when $[S] << K_m$
Data in this table are from reference (23)

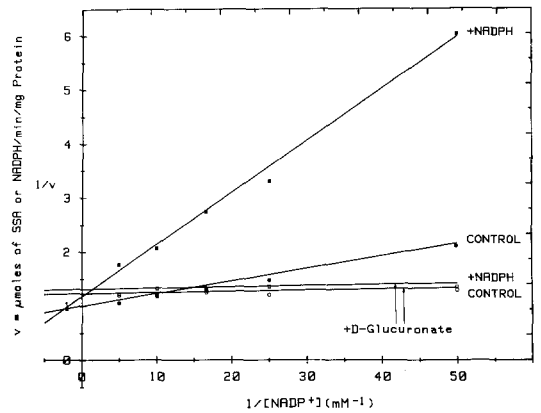

Fig. 3. The effect of D-glucuronate on the kinetics of the oxidation of GHB with $NADP^+$ as the variable substrate (23). In the absence of D-glucuronate, NADPH formation was measured; in the presence of D-glucuronate, succinic semialdehyde (SSA) formation was measured. ● = control, ■ = control with 0.02 mM NADPH, ○ = control with 2.0 mM D-glucuronate, □ = control with 0.02 mM NADPH and 2.0 mM D-glucuronate.

Table II. Kinetic Constants for the Inhibition of GHB-Dehydrogenase (26,30)

Inhibitor	K_i (mM)	
	Uncoupled reaction	Coupled reaction
DL-β-Hydroxybutyrate	3.9	4.4
Acetoacetate	3.0	
α-Ketoglutarate	1.1	0.6
p-Hydroxyphenylpyruvate	1.0	
Phenylacetate	0.5	0.4
Pyruvate	7.0	
α-Ketoisovalerate	0.33	0.2
α-Ketoisocaproate	0.17	
DL-α-keto-β-methyl *n*-Valerate	0.06[a]	
Valproate	0.057	
Salicylate	0.115	

Assays for the coupled reaction and the uncoupled reaction were carried out as described (26).
[a]It should be noted that the K_i has only been reported for the DL-mixture.

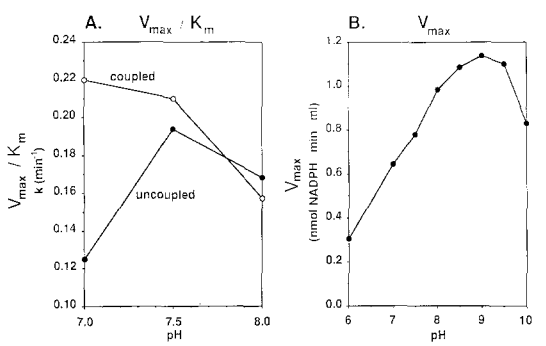

Fig. 4. The effect of pH on the ratio of V_{max}/K_m and on V_{max} for the oxidation of GHB by GHB dehydrogenase. The data for Figure 4A were taken from reference (26). The data in Figure 4B are from reference (19). V_{max}/K_m = the first order rate constant, k, when [S] << K_m.

of the purified enzyme and 2.0 mM DTT inhibits 84% of the activity found in rat liver cytosol. The inhibition produced by DTT can be partially reversed by the addition of H_2O_2 or completely reversed by oxidized glutathione. Preincubation of GHB dehydrogenase with 2.5 mM DTT before it is added to the reaction mixture (and thereby diluted 50 fold) does not inhibit the enzyme. Under these conditions, the sulfhydryl groups are probably reoxidized by molecular oxygen. Little or no stimulatory effect is seen when compounds such as H_2O_2, oxidized glutathione and cystamine, all of which can oxidize sulfhydryl groups, are added to enzyme that has not been *previously* inhibited by sulfhydryl reducing agents.

When the activity of GHB dehydrogenase is being measured, the inclusion of reducing compounds such as β-mercaptoethanol or DTT in the assay mixture as has been reported by some laboratories (27) could lead to incorrect results.

Identification of a Mitochondrial Transhydrogenase Which Catalyzes the α-Ketoglutarate-Dependent Oxidation of GHB. The discovery of GHB dehydrogenase made it possible to complete a sequence of reactions leading from GHB to CO_2 and H_2O. Development of a polyclonal antibody to the GHB dehydrogenase then made it possible to determine whether or not there were additional enzymes in the cytosol or in other subcellular fractions that could catalyze the initial, and probably rate-limiting, step in the oxidation of GHB to CO_2 (31). The antibody, which inhibited ≈ 95% of the ability of the cytosolic fraction to oxidize GHB to SSA, failed to inhibit approximately 60% of this activity in kidney homogenate (Figure 5). These results suggested that there was at least one additional enzyme which catalyzed the conversion of GHB to SSA. The mitochondrial fraction, which had previously been shown to lack GHB dehydrogenase activity (19), could oxidize [^{14}C]GHB to $^{14}CO_2$ and could catalyze the pyridine nucleotide-independent oxidation of GHB to SSA (31). The mitochondrial enzyme was subsequently isolated and shown to be a hydroxyacid-oxoacid transhydrogenase which catalyzed the following reaction (20):

GHB + α-ketoglutarate → SSA + α-hydroxyglutarate.

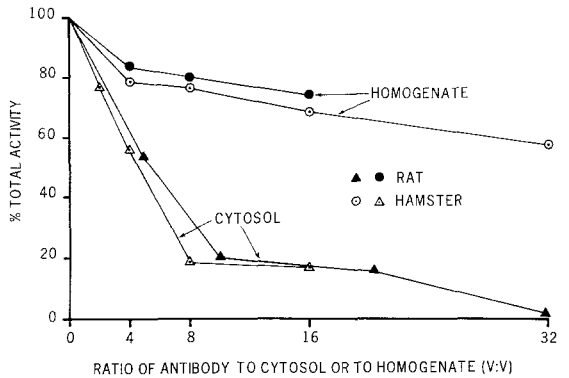

Fig. 5. Titration of GHB dehydrogenase activity in cytosol and homogenate with the antibody to GHB dehydrogenase (31). The conversion of [1-^{14}C]GHB to $^{14}CO_2$ was used as a measure of activity in the experiments in which homogenate was used. When cytosol was used, the conversion of GHB to SSA was measured spectrophotometrically. The cytosol and homogenate used in this experiment were derived from the same amount of kidney. Antibody was added to the reaction mixture in the amounts indicated.

The oxidation of GHB by the mitochondrial enzyme was found to be completely dependent on the presence of α-ketoglutarate. The reaction was reversible. The mitochondrial enzyme also catalyzed the conversion:

α-hydroxyglutarate + SSA → α-ketoglutarate + GHB

with the two products being formed in stoichiometric amounts. The substrate specificity is shown in Table III and the kinetic constants for the principal substrates in Table IV (20). The assumption that this enzyme is a transhydrogenase was confirmed when it was shown by gas chromatographic-mass spectroscopy that a deuterium on the hydroxyl-bearing carbon of one of the optical isomers of DL-γ-deutero-γ-hydroxybutyrate was transferred to the ketone-bearing carbon of α-ketoglutarate. This hydroxyacid-oxoacid transhydrogenase which has been found in the soluble fraction of mitochondria from liver, kidney and brain has been partially purified (20).

Comparison of the Cytosolic and Mitochondrial Enzymes which Oxidize GHB to SSA. With the discovery of the mitochondrial transhydrogenase it became apparent that there was a dual pathway for the initial step in the oxidative pathway for GHB. Both cytosolic GHB dehydrogenase and the mitochondrial enzyme are oxidoreductases, but of remarkably different types. The cytosolic enzyme is an NADP$^+$-dependent dehydrogenase, whereas the mitochondrial enzyme is a pyridine nucleotide-independent, α-ketoglutarate-dependent transhydrogenase. These two enzymes, despite some striking differences, have one property in common: the activity of each of these enzymes is regulated by coupling the oxidation of GHB, the hydroxyacid, to the simultaneous reduction of an oxoacid. Though GHB dehydrogenase can function in the uncoupled state, conditions in the cytosol of most tissues (Table I) would not be favorable to the uncoupled reaction. The mitochondrial enzyme, on the other hand, has an absolute requirement for an oxoacid, which suggests that the metabolism of GHB by the mitochondrial enzyme would depend on the steady state levels of citric acid cycle intermediates and of α-ketoglutarate in particular. The relative activities (V_{max} and v) of these two enzymes in brain and kidney are shown in Table V. The activities of the two enzymes were measured under V_{max} conditions; the rate of GHB oxidation (v) at average tissue GHB concentrations could then be calculated (Table V). How well these values correspond to measurements of GHB catabolism in vivo can be determined by examination of the half-life of GHB in brain. Doherty et al. (16) and Möhler et al. (15) have both reported a half-life ($t_{1/2}$) for labeled GHB in brain of 5 min. If one assumes that the rate of disposal of GHB follows first order kinetics, then from the fol-

Table III. The Relative Rates of the Transhydrogenase Reaction With Other Hydroxyacids and Oxoacids (20)

Hydroxyacid (with SSA as co-substrate)	Relative Rate	*Oxoacid* (with GHB as co-substrate)	Relative Rate
D-α-Hydroxyglutarate	100	α-Ketoglutarate	100
L-α-Hydroxyglutarate	0	α-Ketoadipate	24
DL-β-Hydroxybutyrate	43	Oxalacetate	18.5
L-β-Hydroxybutyrate	45	Pyruvate	8.0
D-β-Hydroxybutyrate	0	α-Ketobutyrate	4.3
DL-α-Hydroxybutyrate	0	Acetoacetate	0
D-Lactate	0	β-Ketoglutarate	0.5
L-Lactate	1.2	β-Ketoadipate	0
D-Malate	0		
L-Malate	0		

The data in this table are derived from reference (20)

Table IV. Kinetic Constants for the Partially Purified Hydroxyacid-Oxoacid Transhydrogenase (20)

Substrate	$K_{m_{app}}$
	M
γ-hydroxybutyrate	3.0×10^{-4}
L-β-hydroxybutyrate	3.0×10^{-3}
α-Hydroxyglutarate	4.2×10^{-4}
α-Ketoglutarate	1.8×10^{-4}
Succinic semialdehyde	4.6×10^{-6}

lowing expression k, the first order rate constant, can be calculated.

$$k = 2.3 \log 2 / t_{1/2}$$

At a brain concentration of 2.3 nmol/g the rate of disposal of GHB, calculated from the $t_{1/2}$ would be 0.32 nmol/min/g. The combined rate of catabolism in brain by the two enzymes was found to be 0.18 nmol/min/g brain (Table V). Since the rate of disposal of GHB calculated from the tissue half-life must include both the rate of catabolism of GHB and the rate of loss from brain by transport to the plasma, the rates calculated from the half-life in brain and from the combined activity of the two catabolic enzymes (Table V) are in reasonable agreement.

Effects of Modulators of GHB Dehydrogenase In Vivo. One approach used to determine the magnitude of the contribution of GHB dehydrogenase to the metabolism of GHB in vivo was to give rats compounds which inhibit the activity of the enzyme in vitro (30). If GHB dehydrogenase plays a significant role in the metabolism of GHB in a tissue, inhibition of the enzyme in vivo would be expected to result in increased tissue concentrations of GHB. Two metabolic intermediates, phenylacetate and α-ketoisocaproate, and two drugs, valproate and salicylate, all potent inhibitors of GHB dehydrogenase with K_i values in the range of 10^{-4} to 10^{-5} M, were administered to rats. The effects of α-ketoisocaproate and phenylacetate (Table VI) and valproate and salicylate (table VII) on tissue contents of GHB varied depending on both the tissue and the compound. *All* of these compounds increased the concentration of GHB in one or more of the tissues assayed, in some cases by 200%. These results are in agreement with those of Snead (32) who found elevated levels of GHB in brain following administration of sodium valproate. Although each of these compounds also inhibits SSA dehydrogenase (30), which catalyzes the second step in the degradative pathway, it is very unlikely that the degree to which this enzyme would be inhibited is sufficient to elevate the concentration of SSA enough to cause the increase in tissue levels of GHB. The values for the K_i for valproate, for GABA transaminase (29) and for SSA dehydrogenase (29,30) are approximately 10^{-2} to 10^{-3}M. These K_i values are several orders of magnitude higher than the K_i for GHB dehydrogenase (30). From these data it was calculated (30) that sodium valproate at a tissue

Table V. Relative Activity of the Degradative Enzymes for GHB in Brain and Kidney

Enzyme	V_{max} nmol/min/gram tissue	K_m (M)	Tissue concentration nmol GHB/ gram tissue	Calculated rate of oxidation of GHB at average tissue concentrations v = nmol/min/gram*
	Brain			
GHB dehydrogenase	10.1	2.5×10^{-3} 4.5×10^{-4}‡	2.3	0.01 0.051 (coupled)
hydroxyacid-oxoacid transhydrogenase	16.6	3×10^{-4}		0.126
	Kidney			
GHB dehydrogenase	324	2.2×10^{-3} 4.5×10^{-4}‡	28.4	4.2 19.2 (coupled)
hydroxyacid-oxoacid transhydrogenase	172	3×10^{-4}		14.9

*The velocity (v) has been calculated from the equation: $v = \frac{V_{max} \cdot [S]}{K_m + [S]}$ where [S] = substrate concentration.

V_{max} data is from reference (41), K_m data are from references (23) and (20), tissue concentrations reference (3).
‡K_m for GHB when the oxidation of GHB is coupled to the reduction of D-glucuronate (23).

Table VI. Effects of α-Ketoisocaproate and of Phenylacetate on Tissue Levels of GHB (30)

Tissue	Saline infusion	α-Ketoisocaproate infusion		Phenylacetate infusion	
	nmol GHB/g tissue	nmol GHB/g tissue	Percent of control	nmol GHB/g tissue	Percent of control
Brain	2.6 ± 0.3 (5)	3.2 ± 0.1 (3)	123	6.1 ± 1.0 (4)[a]	235
Kidney	27.8 ± 3.2 (5)	55.5 ± 8.2 (4)[b]	198	18.4 ± 1.3 (4)[b]	66
Muscle	22.2 ± 3.0 (6)	46.4 ± 9.9 (4)[b]	209	16.7 ± 7.1 (4)	75

Phenylacetate (1.0 M) was given intravenously as an initial bolus of 1.5 ml followed by a constant infusion of ≃ 2.0 ml/h for 2 h. α-Ketoisocaproate (0.5 M) was given intravenously as an initial bolus of 0.6 ml followed by a constant infusion of ≃ 2.0 ml/h for 2 h. At the end of the infusion the animals were killed and the tissues were removed and assayed for GHB as described (30). All values are means ± SEM, numbers of animals are in parentheses.
[a] $p < 0.01$
[b] $p < 0.05$

Table VII. Effects of Salicylate and of Valproate on Tissue Levels of GHB (30)

Tissue	Saline (control)	Salicylate		Valproate	
	nmol GHB/g tissue	nmol GHB/g tissue	Percent of control	nmol GHB/g tissue	Percent of control
Brain	2.9 ± 0.5 (6)	5.8 ± 0.5 (4)[a]	200		
	2.6 ± 0.2 (4)			3.7 ± 0.2 (4)[a]	142
Kidney	34.5 ± 5.7 (6)	52.8 ± 2.5 (5)[b]	153		
	30.0 ± 4.1 (4)			23.7 ± 3.8 (4)	79

Sodium valproate (100 mg/kg i.p.) was given 2 hr prior to decapitation; Sodium salicylate (500 mg/kg i.p.) was given 1 hr prior to decapitation. Tissues were removed and assayed as described (30). All values are means ± SEM, numbers of animals are in parentheses.
[a] $p<0.01$
[b] $p<0.05$

level of 0.3 mM, a reasonable tissue concentration expected in therapeutic use, would produce an 85% inhibition of GHB dehydrogenase but only a 5% inhibition of SSA dehydrogenase and a negligible inhibition of GABA transaminase. It has been reported that sodium valproate inhibits the conversion of SSA to GHB by an aldehyde reductase (EC 1.1.1.2) (29) which may be similar to, or identical to, GHB dehydrogenase (EC 1.1.1.19). However, inhibition of GHB synthesis would not explain the increased tissue levels of GHB seen following administration of sodium valproate.

The kinetic constants which have been determined for GHB dehydrogenase in vitro suggest that, in the uncoupled state, the enzyme would be more likely to synthesize GHB from SSA than to oxidize it. Indeed the identical enzyme, D-glucuronic reductase, is classified as an aldehyde reductase. It is only in the coupled state that the kinetic constants become favorable to the oxidation of GHB catalyzed by this oxidoreductase under in vivo conditions. In kidney, D-glucuronate is likely to be one of the aldehydes coupled to the oxidation of GHB. In this organ *myo*-inositol oxygenase, the enzyme that synthesizes D-glucuronate, has been found to exist as a complex with D-glucuronate reductase (GHB dehydrogenase) and is believed to transfer the newly formed D-glucuronate directly to the oxidoreductase (33). Further proof that this oxidoreductase is probably identical to GHB dehydrogenase came from the finding that 95% of the D-glucuronate reductase activity in kidney cytosol and 80% of that activity in brain cytosol could be titrated with the polyclonal antibody to GHB dehydrogenase (31). What is not known is the extent to which the reaction is coupled in vivo and whether or not there are aldehydes other than D-glucuronate that can serve this role.

To test the extent to which the enzyme is coupled in vivo, the effect of i.v. administration of D-glucuronate on the half-life of [^{14}C]GHB in plasma has been examined (30). The half-life of ^{14}C-labeled GHB in plasma reflects the overall rate of GHB metabolism by the body tissues since urinary loss of the compound is insignificant (34). The effect of the administration of L-gulonate, the product of D-glucuronate oxidation and a competitive

inhibitor, was also examined (30). The results shown in Figure 6 demonstrate that the half-life of GHB in plasma is shortened by D-glucuronate. The ability of D-glucuronate to decrease the half-life of GHB in plasma indicates that GHB dehydrogenase is not completely coupled in vivo. The half-life of GHB in plasma is lengthened by L-gulonate as would be expected from a competitive inhibitor of the enzyme. The effect of inhibitors of GHB dehydrogenase on tissue content and plasma half-life of GHB and the effect of D-glucuronate on the plasma half-life both suggest that this enzyme may play an important role in the metabolism of GHB in vivo.

NADPH is another endogenous inhibitor of GHB dehydrogenase ($K_i = 7 \times 10^{-6}$ M) in the uncoupled state. The results of the experiment in which D-glucuronate was administered to rats have shown that the reaction was not completely coupled since the rate of metabolism could be stimulated by D-glucuronate. In the absence of maximal coupling, changes in the ratio of NADPH/$NADP^+$ such as would occur with low ambient oxygen concentration might be expected to decrease the activity of GHB dehydrogenase and thereby increase the tissue concentration of GHB. The data in Table VIII show that in rats exposed to 5.6% oxygen for 2 hours the concentration of endogenous GHB is increased in brain from 3.0 to 10.7 nmol/gm of brain (a 3.6-fold increase) and in kidney from 24.6 to 57.0 nmol/gm of kidney (a 2.3-fold increase). This change in the redox state would also favor the synthesis of GHB by SSA reductase, an NADPH-dependent enzyme (35).

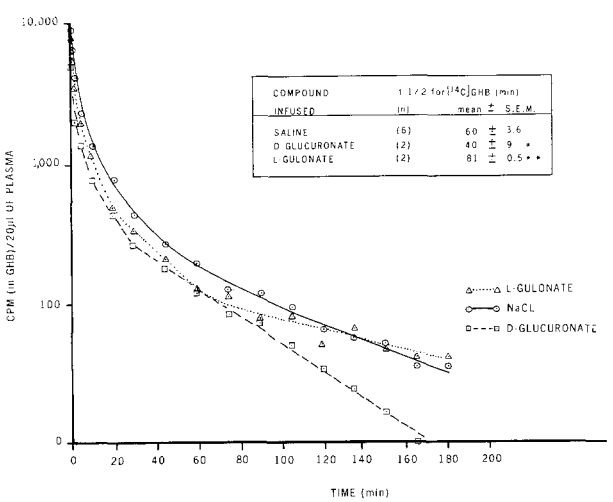

Fig. 6. The effect of D-glucuronate and L-gulonate on the half-life (T½) of GHB in plasma (30). Animals that received D-glucuronate received a bolus containing 240 mg followed by an infusion of 320 mg/h for 3 h. Animals receiving L-gulonate received a bolus of 333 mg and an infusion of 556 mg/h for 3 h. The t½ for the disappearance of [^{14}C]GHB from plasma was determined as described. *$p < 0.05$; **$p < 0.025$.

Table VIII. The Effect of Oxygen Concentration on Tissue Levels of GHB In Vivo

	Normal O_2	Low O_2 (5.6%)	
	nmol GHB/g tissue	nmol GHB/g tissue	Percent of Control
Brain	3.0 ± 0.15 (3)	10.7 ± 0.74 (3)	357*
Kidney	24.6 ± 4.2 (3)	57.0 ± 13.4 (3)	232
Muscle	19.5 ± 4.2 (3)	18.0 ± 2.3 (3)	92

Animals were exposed to either room air or 5.6% oxygen for 2 hr before decapitation. Tissues were removed and assayed as have been described (3). Results are expressed as means ± SEM.
*$p = 0.0005$

Fate of the Carbon Skeleton of GHB. Although GHB is a short chain acid and might be expected to undergo β-oxidation (14), the markedly elevated plasma SSA and GHB concentrations in patients with GHB aciduria (17,18) provide evidence that β-oxidation cannot be the major route of degradation. Inasmuch as the major portion of GHB proceeds through oxidation to SSA, the fate of the SSA formed either by GHB dehydrogenase or the mitochondrial transhydrogenase must be considered. Several investigators have isolated metabolic products formed in vivo after administration of labeled GHB (15,16,27,36–39). The label is distributed in citric acid cycle intermediates and in aspartate, glutamate and GABA. Several reports indicate that the specific activity of GABA is higher than that found in glutamate. Therefore, it has been suggested (37,39) that the GHB skeleton may be converted to GABA by GABA transaminase instead of traversing a more circuitous route leading through the citric acid cycle and glutamate decarboxylase. The work cited above shows that [^{14}C]GHB can label the GABA pool and, in fact, conversion of GHB to GABA has been demonstrated in an in vitro system containing purified GHB dehydrogenase and GABA transaminase (27). However, none of the reported work provides convincing evidence that, in vivo, there is any net flux of the carbon chain of GHB to GABA. In vivo, the SSA formed as a product of the oxidation of GHB by either cytosolic GHB dehydrogenase or the mitochondrial transhydrogenase would encounter not only GABA transaminase but also SSA dehydrogenase. The latter enzyme is an extremely active enzyme with a K_m for SSA of 1.0×10^{-5} M or less (40) and pulls the reaction catalyzed by GABA transaminase in the direction of SSA formation. One would expect that most of the SSA formed in vivo by the enzymatic action of GHB dehydrogenase or by

the mitochondrial transhydrogenase would, like SSA formed by GABA transaminase, be rapidly oxidized to succinate.

SUMMARY

Two enzymes have been found which catalyze the initial step in the catabolism of GHB. The oxidation of GHB to SSA, catalyzed by both of these enzymes, is coupled to the reduction of an oxoacid. In the case of the mitochondrial transhydrogenase, the coupling is obligatory. Although coupling is not obligatory for the GHB dehydrogenase, the stimulation provided by the coupled reaction, and the nature of the kinetics of the uncoupled reaction, may not only allow the reaction to proceed, but may provide a means of regulating the rate of the reaction under in vivo conditions. Since the oxidation of GHB to SSA is the rate limiting step in the overall catabolic pathway (the rate of conversion of GHB to SSA proceeds at approximately one one thousandth of the rate at which SSA is oxidized to succinate by SSA dehydrogenase (30)), factors which regulate the rate of either or both of these enzymes will, in turn, influence tissue levels of endogenous GHB as well as the duration and magnitude of the physiological effect of a dose of GHB.

REFERENCES

1. Roth, R. H., and Giarman, N. J. 1970. Natural occurrence of gamma-hydroxybutyrate in mammalian brain. Biochem. Pharmacol. 19:1087–1093.
2. Roth, R. H. 1970. Formation and regional distribution of γ-hydroxybutyric acid in mammalian brain. Biochem. Pharmacol. 19:3013–3019.
3. Nelson, T., Kaufman, E. E., Kline, J. E., and Sokoloff, L. 1981. The extraneural distribution of γ-hydroxybutyrate. J. Neurochem. 37:1345–18.
4. Winters, W. D., and Spooner, C. F. 1965. A neurophysiological comparison of gamma-hydroxybutyrate with phenobarbital in cats. Electroenceph. Clin. Neurophysiol. 20:83–90.
5. Wolfson, L. I., Sakurada, O., and Sokoloff, L. 1977. Effects of γ-butyrolactone on local cerebral glucose utilization in the rat. J. Neurochem. 29:777–783.
6. Roth, R. H., and Suhr, Y. 1970. Mechanism of the gamma-hydroxybutyrate induced increase in brain dopamine and its relationship to 'sleep'. Biochem. Pharmacol. 19:3001–3012.
7. Laborit, H., Jouany, J.-M., Gerard, J., and Fabiani, F. 1960. Résumé d une étude expérimental et clinique sur un substrat métabolique a action centrale inhibitrice le 4-hydroxybutyrate de Na. Presse Medicale 68:1867–1869.
8. Lavyne, M. H., Hairi, R. J., Tankosic, T., and Babiak, T. 1983. Effect of low dose γ-butyrolactone therapy on forebrain neuronal ischemia in the unrestrained, awake rat. Neurosurg 12:430–434.
9. Boyd, A. J., Sherman, I. A., Saibil, F. G., and Mamelak, M. 1990. The protective effect of γ-hydroxybutyrate in regional intestinal ischemia in the hamster. Gastroenterology 99:860–862.
10. Takahara, J., Yunoki, S., Yakushiji, W., Yamauchi, J., Yamane, Y., and Ofuji, T. 1977. Stimulatory effects of γ-hydroxybutyric acid on growth hormone and prolactin release in humans. J. Clin. Endocrinol. Metab. 44:1014–1017.
11. Yunoki, S. 1982. Studies of gamma-aminobutyric acid (GABA) and its metabolite on the control mechanism of secretion of anterior pituitary hormones. II. Effects of gamma-hydroxybutyric acid (GHB) on secretion of anterior pituitary hormones in human subjects. Okayama Igakkai Zasshi 94:899–913.
12. Kaufman, E. E., Porrino, L. J., and Nelson, T. 1990. Pyretic action of low doses of γ-hydroxybutyrate in rats. Biochem. Pharmacol. 40:2637–2640.
13. Maitre, M., and Mandel, P. 1984. Neurobiology. γ-Hydroxybutyrate, a putative neurotransmitter in the central nervous system. Comptes Rendue Acad. Sci. (Paris) 298 Series III:341–345.
14. Walkenstein, S. S., Wiser, R., Gudmundsen, C., et al. 1964. Metabolism of gamma-hydroxybutyric acid. Biochim. Biophys. Acta 86:640–642.
15. Möhler, H., Patel, A. J., and Balázs, R. 1976. gamma-Hydroxybutyrate degradation in the brain *in vivo*: Negligible direct conversion to GABA. J. Neurochem. 27:253–258.
16. Doherty, J. D., Stout, R. W. and Roth, R. H. 1975. Metabolism of [1-^{14}C]γ-hydroxybutyric acid by rat brain after intraventricular injection. Biochem. Pharmacol. 24:469–474.
17. Jakobs, C., Bojasch, M., Mönch, E., Rating, D., Siemes, H. and Hanefeld, F. 1981. Urinary excretion of gamma-hydroxybutyric acid in a patient with neurological abnormalities. The probability of a new inborn error of metabolism. Clin. Chim. Acta 111:169–178.
18. Gibson, K. M., Sweetman, L., Nyhan, W. L., Jakobs, C., Rating, D., Siemes, H. and Hanefeld, F. 1981. Succinic semialdehyde dehydrogenase deficiency: An inborn error of gamma-aminobutyric acid metabolism. Clin. Chim. Acta 133:33–42.
19. Kaufman, E. E., Nelson, T., Goochee, C. F., and Sokoloff, L. 1979. Purification and characterization of an NADP$^+$-linked alcohol oxido-reductase which catalyzes the interconversion of γ-hydroxybutyrate and succinic semialdehyde. J. Neurochem. 32:699–712.
20. Kaufman, E. E., Nelson, T., Fales, H. M., and Levin, D.M. 1988. Isolation and characterization of a hydroxyacid-oxoacid transhydrogenase from rat kidney mitochondria. J. Biol. Chem. 263:16872–16879.
21. York, J. L., Grollman, A. P. and Bublitz, C. 1961. TPN-L-gulonate dehydrogenase. Biochim. Biophys. Acta 47:298–306.
22. Cromlish, J. A., Yoshimoto, C. K., and Flynn, G. T. 1985. Purification and characterization of four NADPH-dependent aldehyde reductases from pig brain. J. Neurochem. 44:1477–1484.
23. Kaufman, E. E., and Nelson, T. 1981. Kinetics of coupled γ-hydroxybutyrate oxidation and D-glucuronate reduction by an NADP$^+$-dependent oxidoreductase. J. Biol. Chem. 256:6890–6894.
24. Segel I.H. 1975. Page 41. Enzyme Kinetics. Wiley Interscience, New York.
25. Glock, G. E. 1961. Long, C. (ed.), Biochemist's Handbook, Van Nostrand, Princeton.
26. Kaufman, E. E., Relkin, N., and Nelson, T. 1983. Regulation and properties of an NADP$^+$ oxidoreductase which functions as a γ-hydroxybutyrate dehydrogenase. J. Neurochem. 40:1639–1646.
27. Vayer, P., Schmitt, M., Bourguignon, J. J., Mandel, P. and Maitre, M. 1985. Evidence for a role of high K_m aldehyde reductase in the degradation of endogenous γ-hydroxybutyrate from rat brain. FEBS LETTERS 190:55–60.
28. Erwin, V. G., and Deitrich, R. A. 1973. Inhibition of bovine brain aldehyde reductase by anticonvulsant compounds *in vitro*. Biochem. Pharmacol. 22:2615–2624.
29. Whittle, S. R., and Turner, A. J. 1978. Effects of the anticonvulsant sodium valproate on γ-aminobutyrate and aldehyde metabolism in ox brain. J. Neurochem. 31:1453–1459.
30. Kaufman, E. E., and Nelson, T. 1987. Evidence for the participation of a cytosolic NADP$^+$-dependent oxidoreductase in the

catabolism of γ-hydroxybutyrate *in vivo*. J. Neurochem. 48:1935–1941.
31. Kaufman, E. E., Nelson, T., Miller, D., and Stadlan, N. 1988. Oxidation of γ-hydroxybutyrate to succinic semialdehyde by a mitochondrial pyridine nucleotide-independent enzyme. J. Neurochem. 51:1079–1084.
32. Snead, O. C., III, Bearden, L. J., and Pegram, V. 1980. Effect of acute and chronic anticonvulsant administration on endogenous γ-hydroxybutyrate in rat brain. Neuropharmacology 19:47–52.
33. Reddy, C. C., Swan, J. S., and Hamilton, G. A. 1981. myo-Inositol oxygenase from hog kidney: 1. Purification and characterization of an enzyme complex containing the oxygenase and D-glucuronate reductase. J. Biol. Chem. 256:8510–8518.
34. Vickers, M. D. 1969. Gammahydroxybutyric Acid. Int. Anesthesiol. Clin. 7:75–89.
35. Rumigny, J. F., Cash, C., Mandel, P., Vincendon, G. and Maitre, M. 1981. Evidence that a specific succinic semialdehyde reductase is responsible for γ-hydroxybutyrate synthesis in rat brain tissue slices. FEBS LETTERS 134:96–98.
36. Mitoma, C., and Neubauer, S. E. 1968. gamma-Hydroxybutyric acid and sleep. Experientia 24:12–13.
37. DeFeudis, F. V., Delgado, J. M., and Roth, R. H. 1969. Content and release of amino-acids and catecholamines in monkey brain. Nature 223:74–75.
38. DeFeudis, F. V., and Collier, B. 1970. Amino acids of brain and γ-hydroxybutyrate-induced depression. Archs. Int. Pharmacodyn. Thér. 187:30–36.
39. Vayer, P., Mandel, P., and Maitre, M. 1985. Conversion of γ-hydroxybutyrate to γ-aminobutyrate *in vitro*. J. Neurochem. 45:810–814.
40. Baxter, C. F. 1970. The nature of γ-aminobutyric acid. Pages 289–53, *in* Lajtha, A. (ed.), Handbook of Neurochemistry Volume 3, Plenum, New York.
41. Kaufman, E. E., Nelson, T., and Fales, H. M. 1990. Isolation and characterization of a mammalian hydroxyacid-oxoacid transhydrogenase. Pages 1059–1069, in, Reddy, C. C., Hamilton, G. A. (eds.), Proceeding of the International Symposium on Biological Oxidation Systems. Volume II, Academic Press, New York.