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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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(Accepted April 22, 1991)

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 CO₂ and water. These investigators could not find the ¹⁴C 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 [¹⁴C]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 $\rightarrow \rightarrow \rightarrow$ Co₂ + H₂O

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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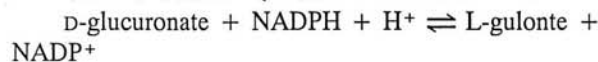
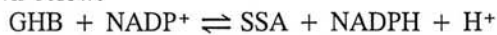
* Special issue dedicated to Dr. Louis Sokoloff.

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

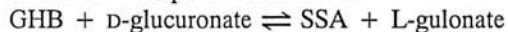
dehydrogenase (the enzyme missing in patients with GHB aciduria (18)) catalyzed the reaction in step (2). Step (1) is always depicted as an essential part of this scheme. However, at the time this pathway was proposed, an enzyme or enzymes which could catalyze this step had not been identified.

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-glucuronate 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:



The overall or "coupled" reaction is:



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

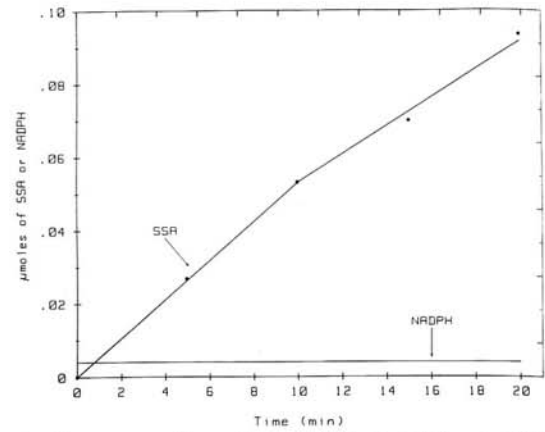


Fig. 1. Time course of succinic semialdehyde (SSA) and NADPH formation in the presence of D-glucuronate (23). The reaction mixture contains 10 mM GHB, 1.0 mM D-glucuronate, 0.1 mM NADP⁺, 80 mM phosphate buffer, pH 7.6, enzyme and sufficient water to bring the volume to 1.0 ml. SSA and NADPH were determined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

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.

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-

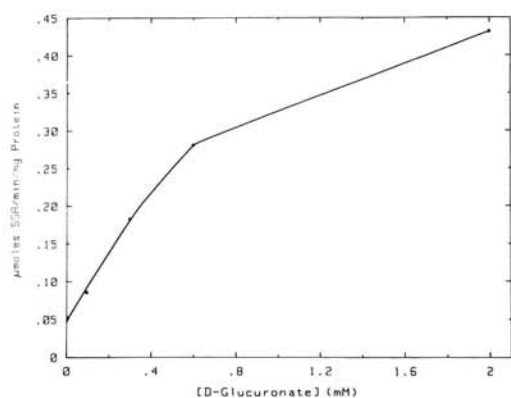


Fig. 2. The effect of D-glucuronate concentration on the rate of conversion of GHB to succinic semialdehyde (SSA) in a reaction mix containing NADPH and a low concentration of NADP⁺ (23). Reaction mixture: 10 mM GHB, 0.01 mM NADP⁺, 0.02 mM NADPH, 80 mM phosphate, pH 7.6, enzyme, D-glucuronate as indicated 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 oxido-reductase 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_{mapp}	2.3×10^{-3} M	4.5×10^{-4} M ^b	$0.1-5 \times 10^{-5}$ M
NADP ⁺	K_{mapp}	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	

^aThe 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).

^b1 mM D-glucuronate.

^c2 mM D-glucuronate.

^d μ mol/min/mg protein.

^efirst order rate constant when $[S] \ll K_m$

Data in this table are from reference (23)

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