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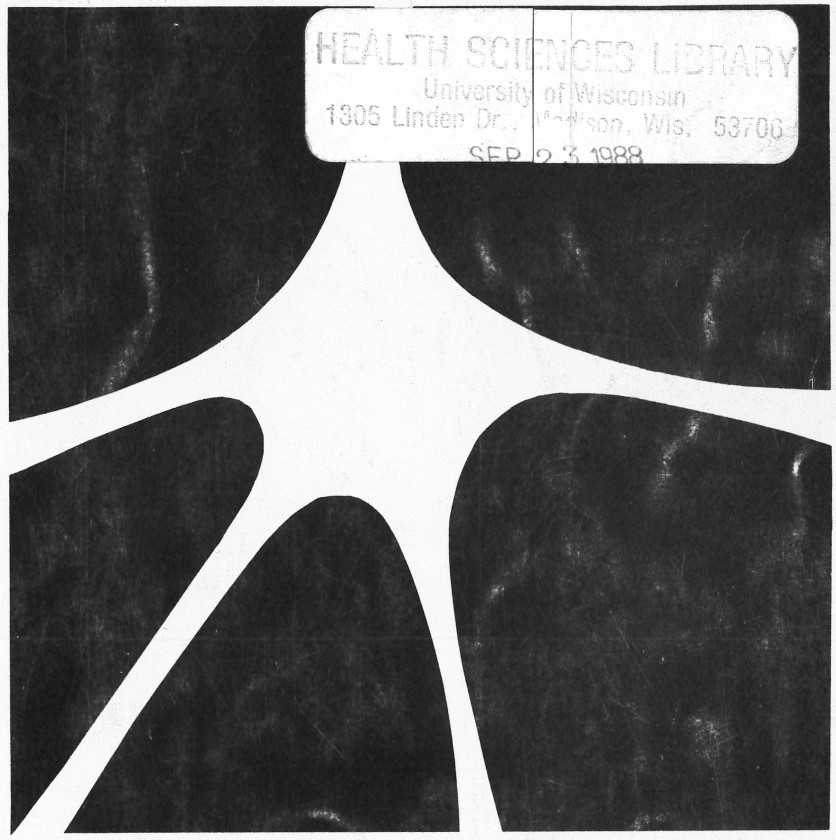

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Oxidation of γ-Hydroxybutyrate to Succinic Semialdehyde by a Mitochondrial Pyridine Nucleotide-Independent Enzyme

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Abstract: An antibody that inhibits over 95% of the cytosolic $NADP^+$-dependent γ-hydroxybutyrate (GHB) dehydrogenase activity of either rat brain or kidney was found to inhibit only approximately 50% of the conversion of [1-^{14}C]GHB to $^{14}CO_2$ by rat kidney homogenate. A similar result was obtained with sodium valproate, a potent inhibitor of GHB dehydrogenase. The mitochondrial fraction from rat brain and kidney was found to catalyze the conversion of [1-^{14}C]GHB to $^{14}CO_2$. The dialyzed mitochondrial fraction also catalyzed the oxidation of GHB to succinic semialdehyde (SSA) in a reaction that did not require added NAD^+ or $NADP^+$ and which was not inhibited by sodium valproate. The enzyme from the mitochondrial fraction which converts GHB to SSA appears to be distinct from the $NADP^+$-dependent cytosolic oxidoreductase which catalyzes this reaction.
Key Words: γ-Hydroxybutyrate—Succinic semialdehyde—Mitochondria—Valproate—Rat brain. **Kaufman E. E. et al.** Oxidation of γ-hydroxybutyrate to succinic semialdehyde by a mitochondrial pyridine nucleotide-independent enzyme. *J. Neurochem.* **51,** 1079–1084 (1988).

It has been known for some time that the neuroactive compound γ-hydroxybutyrate (GHB) is metabolized to CO_2 and water (Walkenstein et al., 1964) and that, in its major degradative pathway, the carbon skeleton of GHB enters the citric acid cycle as succinate rather than as acetyl-CoA (Doherty et al., 1975; Möhler et al., 1976). The initial step in this pathway, the oxidation of GHB to succinic semialdehyde (SSA), has been investigated only recently. In 1979, we reported a cytosolic $NADP^+$-dependent oxidoreductase (Kaufman et al., 1979) that catalyzes this reaction. It is now possible to write a complete sequence of reactions leading from GHB to CO_2 and to identify an enzyme capable of catalyzing each of the reactions. However, it is still not known whether there are other cytosolic oxidoreductases or enzymes in other subcellular compartments that can oxidize GHB to SSA. The development of an antibody to the cytosolic GHB dehydrogenase and the identification of several good inhibitors of this enzyme have led to the disclosure of a second enzyme capable of catalyzing this reaction.

MATERIALS AND METHODS

Materials and animals

Sprague–Dawley male rats weighing 350–450 g were obtained from Taconic Farms (Germantown, NY, U.S.A.). SSA and sodium GHB were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Valproic acid was obtained from Saber Laboratories (Morton Grove, IL, U.S.A.). [1-^{14}C]GHB (37 mCi/mmol) was obtained from Research Products International Corp. (Mount Prospect, IL, U.S.A.). Center well incubation flasks with rubber stoppers and disposable plastic wells were obtained from Kontes Scientific Glassware (Vineland, NJ, U.S.A.).

Assay procedures

Unless otherwise stated, the oxidation of GHB to SSA catalyzed by purified cytosolic GHB dehydrogenase or by cytosol was assayed spectrophotometrically at 37°C as described by

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Abbreviations used: GHB, γ-hydroxybutyrate; PCA, perchloric acid; SSA, succinic semialdehyde.

Kaufman et al. (1979) in a reaction mixture containing 0.08 *M* potassium phosphate (pH 7.6), 0.0025 *M* $NADP^+$, purified enzyme or cytosol, 0.01 *M* sodium GHB, and sufficient water to bring the volume to 1.0 ml. Additions of antibody or inhibitor are noted in the legends to the figures.

In experiments in which D-glucuronate reductase or SSA reductase activity in cytosol was measured, the reaction mixture contained 0.2 m*M* NADPH, 80 m*M* phosphate (pH 7.0), the enzyme fraction to be assayed, 1.0 m*M* SSA or 10 m*M* D-glucuronate, and sufficient water to bring the volume to 1.0 ml. The reaction was followed by measurement of the change of absorbance at 340 nm with time.

In experiments in which homogenate or mitochondria were used as a source of enzyme, the oxidation of GHB was followed by measuring the oxidation of [1-^{14}C]GHB to $^{14}CO_2$. These experiments were carried out in 10-ml Erlenmeyer flasks equipped with serum caps and hanging plastic wells. A 1-cm^2 piece of filter paper wetted with 100 μl of 1.0 *M* KOH was placed in each hanging well just prior to the start of the experiment. The reaction was started by the addition of [1-^{14}C]GHB, and at the appropriate time was stopped by the injection through the serum cap of 200 μl of 15% perchloric acid (PCA) containing 1.0 *M* GHB. The flasks were incubated for an additional 60 min at 37°C to insure complete release of the $^{14}CO_2$ from the reaction mixture. The caps were then removed, and the center wells containing the filter paper squares were placed in counting vials with 1 ml of water and 10 ml of Aquasol (New England Nuclear, Boston, MA, U.S.A.), and counted in a Packard Tri-Carb scintillation counter (model no. 3375; Packard Instrument Co., Downers Grove, IL, U.S.A.).

Assays in which the formation of [^{14}C]SSA from [1-^{14}C]GHB was measured in the mitochondrial fraction were carried out as follows. A large pool of SSA (5 m*M* final concentration) was added to the test tubes containing the reaction mixture to trap the [^{14}C]SSA formed from [1-^{14}C]GHB. The reaction was stopped by the addition of 50 μl of 60% PCA, the precipitated protein was spun down, and the supernatant phase was neutralized by the addition of 50 μl of 5 *M* K_2CO_3. Two micromoles of SSA were then added to a 0.6-ml portion of the neutralized supernatant fraction, followed by 800 μl of a 2% solution of 2,4-dinitrophenylhydrazine, to form the insoluble 2,4-dinitrophenylhydrazone of SSA. The test tubes were allowed to stand at 21°C in the dark for 3 h while the precipitate formed. The precipitate was collected on 0.5-cm filters and washed three times with 0.2-ml portions of glass-distilled water. The filters were then placed in small glass tubes and 2 ml of absolute ethanol added to dissolve the precipitated 2,4-dinitrophenylhydrazone. One milliliter of this solution was added to counting vials containing 10 ml of Aquasol, and the [^{14}C]SSA was assayed in a Beckman LS 5801 scintillation counter with automatic quench correction. To correct for any loss of SSA due to metabolism, a portion of the neutralized supernatant fraction from the zero time sample and from each incubated sample was assayed for SSA content as previously described (Kaufman et al., 1979). The amount of [^{14}C]SSA formed and trapped in the large pool of nonradioactive SSA was calculated from the ^{14}C found in the SSA and the known specific activity of the [^{14}C]GHB. This assay was linear with time up to 20 min and gave results which agreed with those obtained with the $^{14}CO_2$ assay.

Preparation of tissue homogenates, subcellular fractions, and enzymes

Subcellular fractions were separated by differential centrifugation as described by Sokoloff and Kaufman (1961). The method of Yonetani (1967) was used to assay the various subcellular fractions for cytochrome oxidase activity, a common mitochondrial marker. The method of Wallenfels (1962) was used to assay these fractions for β-galactosidase, a lysosomal marker. Tissue homogenates were also prepared as described by Sokoloff and Kaufman (1961). Hamster liver GHB dehydrogenase was prepared and purified as previously described (Kaufman et al., 1979).

Antibody preparation

Antibody to purified hamster liver GHB dehydrogenase was produced in a goat by repeated intracutaneous injections of a mixture of Freund's adjuvant and purified enzyme over a period of several months. Antibody formation was monitored by determination of inhibition of GHB dehydrogenase activity by samples of goat plasma. After a sufficiently high titer of anti-GHB dehydrogenase activity had been achieved, plasma was obtained from the goat by plasmapheresis. Partial purification of the antibody was obtained by adding an equal volume of saturated $(NH_4)_2SO_4$ to the plasma at 21°C and centrifuging to spin down the precipitated antibody. The pellet, containing the antibody activity, was dissolved in a volume of 0.9% saline equal to that of the original plasma. The $(NH_4)_2SO_4$ step was repeated and the resulting precipitate was redissolved in phosphate-buffered saline, pH 7.2; the residual $(NH_4)_2SO_4$ was removed by dialysis against phosphate-buffered saline. The resulting antibody fraction was used for subsequent studies.

RESULTS

Titration of GHB dehydrogenase activity in rat brain and rat kidney cytosol with antibody to purified GHB dehydrogenase

The antibody to the purified hamster liver GHB dehydrogenase was used to titrate the GHB dehydrogenase activity in the cytosol from rat brain and rat kidney (Fig. 1B and C). A titration curve for the purified enzyme (Fig. 1A) is included for comparison. The $NADP^+$-dependent GHB dehydrogenase activity in both brain and kidney cytosol, like the purified enzyme, was essentially completely inhibited by the antibody. Rat kidney cytosol also contains a very small amount of NAD^+-dependent GHB dehydrogenase activity (less than 5% of the $NADP^+$-dependent activity). This activity was not inhibited by the antibody and contributes very little to the overall catabolism of GHB. These results suggest that there is only one major GHB dehydrogenase, i.e., the $NADP^+$-dependent GHB dehydrogenase (Kaufman et al., 1979), a cytosolic enzyme found in both rat brain and kidney.

Because the $NADP^+$-dependent GHB dehydrogenase can also function as a SSA reductase and as a D-glucuronate reductase (Kaufman and Nelson, 1981), the ability of the antibody to titrate these activities was also tested. In brain, approximately 80% of these activities could be inhibited, whereas in kidney, approximately 90% of the SSA reductase and 95% of the D-glucuronate reductase activity could be inhibited. The data presented in Fig. 1B suggest that approximately 20% of the SSA reductase activity in brain cytosol is due to other enzymes. This finding agrees with that of

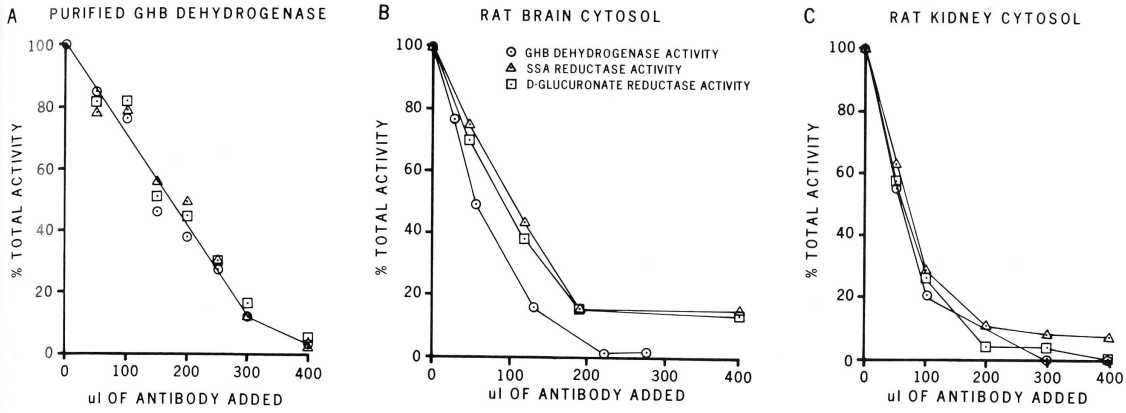

FIG. 1. Titration curves for purified GHB dehydrogenase (**A**), rat brain cytosol (**B**), and rat kidney cytosol (**C**). The rates of oxidation of GHB and of reduction of SSA and D-glucuronate were measured spectrophotometrically as described under Materials and Methods. Additions of antibody to the reaction mixture were as indicated in the figure.

other investigators (Tabakoff and Von Wartburg, 1975; Wermuth et al., 1977; Rumigny et al., 1980).

Comparison of the inhibitory effects of sodium valproate on purified GHB dehydrogenase and on GHB dehydrogenase activity in rat brain cytosol

Sodium valproate, which is a good inhibitor ($K_i = 5.7 \times 10^{-5}$ *M*) of GHB dehydrogenase (Kaufman and Nelson, 1987), was also used to titrate the activity of this enzyme in brain cytosol. At 5 m*M* sodium valproate, 95% of the activity of the purified enzyme was inhibited, as was approximately 92% of the activity in the brain cytosol (Fig. 2). The results obtained with sodium valproate (Fig. 2) are in good agreement with those obtained with the antibody as shown in Fig. 1A.

The inhibitory effect of the antibody to GHB dehydrogenase on GHB oxidation in kidney homogenate and cytosol

Although the titration curves shown in Figs. 1 and 2, as well as the very low amount of pyridine nucleotide-dependent activity seen with cytosol and NAD^+ (see above), provide evidence that the $NADP^+$-dependent GHB dehydrogenase is the major dehydrogenase in cytosol catalyzing the conversion of GHB to SSA, they do not indicate whether there are additional enzymes that catalyze this reaction in other subcellular compartments. To answer this question, we again used the antibody to GHB dehydrogenase to titrate the conversion of [1-^{14}C]GHB to $^{14}CO_2$ in whole kidney homogenates. Because it has been established that the catabolic pathway from GHB to CO_2 proceeds through SSA and succinate to the citric acid cycle, titration of the unfractionated homogenate should provide an estimate of the role of the cytosolic GHB dehydrogenase in the overall catabolism of GHB. As we have pointed out previously (Kaufman and Nelson, 1987), the activity of GHB dehydrogenase is severalfold lower than that of the enzymes which catalyze the subsequent steps in the pathway leading from GHB to CO_2, i.e., SSA dehydrogenase and the enzymes of the citric acid cycle. If GHB dehydrogenase were the only enzyme capable of catalyzing the initial step in this pathway, inhibition of this rate-limiting enzyme should result in a similar inhibition of the entire pathway. The production of $^{14}CO_2$ from [1-^{14}C]GHB in the presence of homogenate should be inhibited to approximately the same extent by the addition of antibody as is the $NADP^+$-dependent oxidation of GHB by the cytosolic GHB dehydrogenase. As can be seen from Fig. 3, whereas approximately 95% of the GHB dehydrogenase activity in the cytosol can be inhibited with the antibody, only a little

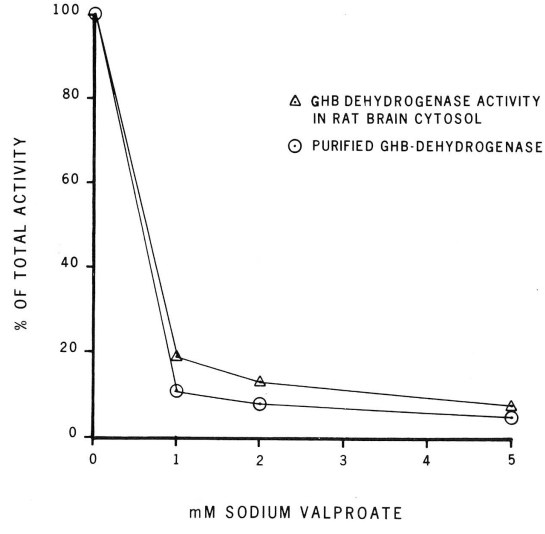

FIG. 2. Titration of GHB dehydrogenase activity in rat brain cytosol with sodium valproate. The rate of GHB oxidation was measured as described under Materials and Methods. Sodium valproate was added to the reaction mixture to give concentrations of 0, 1, 2, and 5 m*M*.

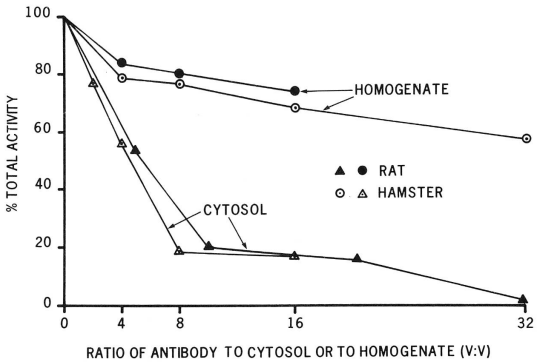

FIG. 3. Titration of GHB dehydrogenase activity in cytosol and homogenate with the antibody to GHB dehydrogenase. The conversion of [1-^{14}C]GHB to $^{14}CO_2$ was used as a measure of activity in the experiments in which homogenate was used. When cytosol was used, the conversion of GHB to SSA was measured 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.

more than 40% of the overall conversion of [1-^{14}C]GHB to $^{14}CO_2$ can be inhibited in the whole homogenate. These results strongly suggest that there is at least one additional enzyme that can catalyze the conversion of GHB to SSA.

Comparison of the abilities of tissue homogenates and the mitochondrial fraction to catalyze conversion of [1-^{14}C]GHB to $^{14}CO_2$

It is known that the enzymes required to catalyze the reactions leading from SSA to CO_2, i.e., SSA dehydrogenase and the enzymes of the citric acid cycle, are all mitochondrial enzymes. A GHB dehydrogenase, that can catalyze the first reaction in the catabolic pathway, has been found previously in the cytosol, but not in mitochondria or other subcellular fractions (Kaufman et al., 1979). To determine whether or not a mitochondrial fraction also contains an enzyme that can catalyze the first reaction in the catabolic pathway, we examined the ability of washed mitochondrial fractions from brain, kidney, and liver to catalyze the conversion of [1-^{14}C]GHB to $^{14}CO_2$. The results shown in Table 1 demonstrate that the mitochondrial fraction alone can carry out the whole catabolic sequence, indicating, therefore, that this fraction also contains an enzyme that can oxidize GHB to SSA. When compared to the homogenate (on a tissue weight basis), the ability of the mitochondrial fraction to convert [1-^{14}C]GHB to $^{14}CO_2$ ranges from approximately 31% in kidney to 54% in liver. The activity of the new GHB oxidizing enzyme was found to distribute in the same manner as does cytochrome oxidase (manuscript in preparation).

The distribution of the new GHB oxidizing enzyme was also compared to that of β-galactosidase, an enzyme used as a lysosomal marker. All of the β-galactosidase activity present in the tissue homogenate could be recovered in the supernatant fraction from the centrifugation step used to obtain the mitochondrial pellet; none was present in the resuspended mitochondrial pellet either before or after freezing and thawing. These data support a mitochondrial localization for the new GHB oxidizing enzyme.

The measurement of the conversion of [1-^{14}C]GHB to [^{14}C]SSA by rat brain mitochondria

The work of both Doherty et al. (1975) and Möhler et al. (1976) has established that the major portion of the carbon skeleton of GHB enters the citric acid cycle as succinate. The implication of this work is that the preceding steps are the oxidation of GHB to SSA, followed by the oxidation of SSA to succinate. For this reason, we looked for a method of measuring SSA formation in a mitochondrial preparation. The trapping of [^{14}C]SSA, formed as a result of the oxidation of [1-^{14}C]GHB, in a large pool of unlabeled SSA with the subsequent isolation of the SSA as the insoluble 2,4-dinitrophenylhydrazone proved to be a satisfactory method. If one assumes that the conversion of GHB to SSA is the rate-limiting step in the conversion of GHB to CO_2, then the amounts of SSA and CO_2 formed should be equal. As can be seen from Tables 1 and 2, this is the case for rat brain mitochondria.

This method was used to determine whether the oxidation of GHB to SSA by the mitochondrial enzyme was dependent on NAD^+ and $NADP^+$. In the first column of Table 2, the results of experiments with disrupted mitochondria with and without either NAD^+ or $NADP^+$ are shown. No dependence on these nucleotides was evident. Because the mitochondrial preparation could have contained endogenous cofactor, the mitochondrial preparations were extensively dialyzed and reassayed. The results obtained with the dialyzed preparation are shown in the second column

TABLE 1. *Distribution of the capacity of tissue fractions to oxidize [1-^{14}C]GHB to $^{14}CO_2$*

Tissue	Fraction	nmol of $^{14}CO_2$/min/g of tissue	% of total activity in homogenate
Liver	Homogenate	23.0	
	Mitochondria	12.4	54%
Kidney	Homogenate	28.7	
	Mitochondria	8.9	31%
Brain	Homogenate	3.9	
	Mitochondria	1.7	44%

The conversion of [1-^{14}C]GHB to $^{14}CO_2$ was measured as described in Materials and Methods. Homogenates were prepared in 0.25 *M* sucrose. The reaction mix contained 2 m*M* D-glucose, 2 m*M* D-glucuronate, 3 m*M* $MgCl_2$, 5 m*M* ADP, 12 m*M* phosphate (pH 7.4), 100 m*M* HEPES (pH 7.4), 200 m*M* sucrose, 2 m*M* $NADP^+$, and 50 μl of liver or kidney mitochondria. Where brain homogenate or mitochondria were used, 200 μl were added. Sufficient water to bring the volume to 1.0 ml was added.

of Table 2. Again, there is no dependence on either added NAD^+ or added $NADP^+$. Although oxidation of GHB to CO_2 by undialyzed mitochondria, illustrated in Table 2, appears to be lower in the presence of $NADP^+$ or NAD^+ than in the absence of these pyridine nucleotides, this effect was not seen consistently. Moreover, neither NAD^+ nor $NADP^+$ had a significant effect on the rate of oxidation of GHB catalyzed by dialyzed mitochondria. This result is in agreement with our earlier failure to detect any significant NAD^+- or $NADP^+$-dependent GHB dehydrogenase activity in any fraction other than cytosol (Kaufman et al., 1979).

The effect of sodium valproate on the conversion of [1-^{14}C]GHB to [^{14}C]SSA by rat kidney mitochondria

Almost 95% of the cytosolic, $NADP^+$-dependent GHB dehydrogenase can be inhibited by sodium valproate (Fig. 2). In contrast, when the disrupted rat kidney mitochondrial fraction was assayed with 2 m*M* sodium valproate, there was no inhibition of the oxidation of [1-^{14}C]GHB to [^{14}C]SSA (Table 3).

DISCUSSION

The experiments described in this paper were designed to answer the question of whether, in addition to the cytosolic $NADP^+$-dependent GHB dehydrogenase, there are other enzymes in either the cytosol or other subcellular fractions that can oxidize GHB to SSA, the first reaction in the catabolic pathway leading to CO_2 and water.

The data presented in Figs. 1 and 2 demonstrate that the $NADP^+$-dependent oxidation of GHB catalyzed by either brain or kidney cytosol can be inhibited almost completely by an antibody to GHB dehydrogenase and, in the case of brain cytosol, also by sodium valproate. Because there is only a trivial amount of NAD^+-dependent activity in cytosol, the $NADP^+$-dependent GHB dehydrogenase appears to be the predominant pyridine nucleotide-dependent dehydrogenase capable of oxidizing GHB in cytosol.

Failure to inhibit nearly 60% of the ability of rat or hamster kidney homogenate to convert [1-^{14}C]GHB to $^{14}CO_2$ led us to examine the possibility that the mitochondrial fraction might also catalyze this complete sequence of reactions. We found that (1) the mitochondrial fraction can catalyze the conversion of [1-^{14}C]GHB to [^{14}C]SSA, and (2) conversion of [1-^{14}C]GHB to $^{14}CO_2$ by the mitochondrial fraction could be demonstrated. The enzyme found in this subcellular fraction appears to be distinct from the cytosolic enzyme in that it does not require either added NAD^+ or $NADP^+$ and is not inhibited by sodium valproate. As is shown in Table 1, the fraction of the total catabolic pathway found in the mitochondria is approximately 30% in kidney, 50% in liver, and 44% in brain.

On the basis of these experiments, we conclude that there are at least two enzymes that can catalyze the first reaction in the catabolic pathway for GHB, i.e., the oxidation of GHB to SSA. One of these enzymes is a cytosolic $NADP^+$-dependent oxidoreductase (Kaufman et al., 1979), whereas the other is present in the mitochondrial fraction and does not require NAD^+ or $NADP^+$. We are now attempting to isolate and characterize this pyridine nucleotide-independent oxidoreductase.

TABLE 2. *Conversion of [1-^{14}C]GHB to [^{14}C]SSA by rat brain mitochondria*

Added cofactors	nmol SSA formed/min/g brain[a]	
	Undialyzed mitochondria	Dialyzed mitochondria[b]
None	2.1	1.2
2.5 m*M* $NADP^+$	1.5	1.3
2.5 m*M* NAD^+	1.3	1.1

The reaction was carried out as described under Materials and Methods. This experiment was repeated five times, and the difference between the control flasks and the flasks containing NAD^+ was not significant.

[a] This rate was linear with time for 20 min. The reaction mix contained 0.1 *M* HEPES (pH 7.0), 5 m*M* phosphate (pH 7.4), 3 m*M* $MgCl_2$, 5 m*M* SSA, 0.25 m*M* [1-^{14}C]GHB (sp. act. = 1,000 cpm/nmol), 50 μl of mitochondrial resuspension, and sufficient water to bring the volume to 1.0 ml.

[b] Approximately 2.0 ml of resuspended, four times frozen-thawed mitochondria were dialyzed two times against 2 L of 0.25 *M* sucrose to remove endogenous cofactors.

TABLE 3. *Effect of sodium valproate on the conversion of [1-^{14}C]GHB to [^{14}C]SSA by rat kidney mitochondria*

Additions to reaction mix	nmol SSA formed/min/g of kidney
Experiment 1	
None	1.78
2 m*M* valproate	1.74
Experiment 2	
None	2.16
2 m*M* valproate	2.21

The reactions were carried out as described under Materials and Methods.

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REFERENCES

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.

Kaufman E. E. and Nelson T. (1981) Kinetics of coupled γ-hydroxybutyrate oxidation and D-glucuronate reduction by an $NADP^+$-oxidoreductase. *J. Biol. Chem.* **256,** 6890–6894.

Kaufman E. E. and Nelson T. (1987) Evidence for the participation of a cytosolic $NADP^+$-dependent oxidoreductase in the catab-

olism of γ-hydroxybutyrate in vivo. *J. Neurochem.* **48,** 1935–1941.

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.

Kaufman E. E., Nelson T., Goochee C., 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.

Möhler H., Patel A. J., and Balazs R. (1976) Gamma-hydroxybutyrate degradation in the brain in vivo: negligible direct conversion to GABA. *J. Neurochem.* **27,** 253–258.

Rumigny J. F., Maitre M., Cash C., and Mandel P. (1980) Specific and non-specific succinic semialdehyde reductases from rat brain: isolation and properties. *FEBS Lett.* **117,** 111–116.

Sokoloff L. and Kaufman S. (1961) Thyroxine stimulation of amino acid incorporation into protein. *J. Biol. Chem.* **236,** 795–803.

Tabakoff B. and Von Wartburg J. P. (1975) Separation of aldehyde reductases and alcohol dehydrogenases from brain by affinity chromatography: metabolism of succinic semialdehyde and ethanol. *Biochem. Biophys. Res. Commun.* **63,** 957–966.

Walkenstein S., Wiser R., Gudmundsen C., and Kimel H. (1964) Metabolism of γ-hydroxybutyric acid. *Biochim. Biophys. Acta* **86,** 640–642.

Wallenfels K. (1962) β-Galactosidase (crystalline), in *Methods in Enzymology, Vol. V* (Colowick S. P. and Kaplan N. O., eds), pp. 212–213. Academic Press, New York.

Wermuth B., Munch J. D. B., and Von Wartburg J. P. (1977) Purification and properties of NADPH-dependent aldehyde reductase from human liver. *J. Biol. Chem.* **252,** 3821–3828.

Yonetani T. (1967) Cytochrome oxidase: beef heart, in *Methods in Enzymology, Vol. X* (Estabrook R. W. and Pullman M. E., eds), pp. 332–335. Academic Press, New York.