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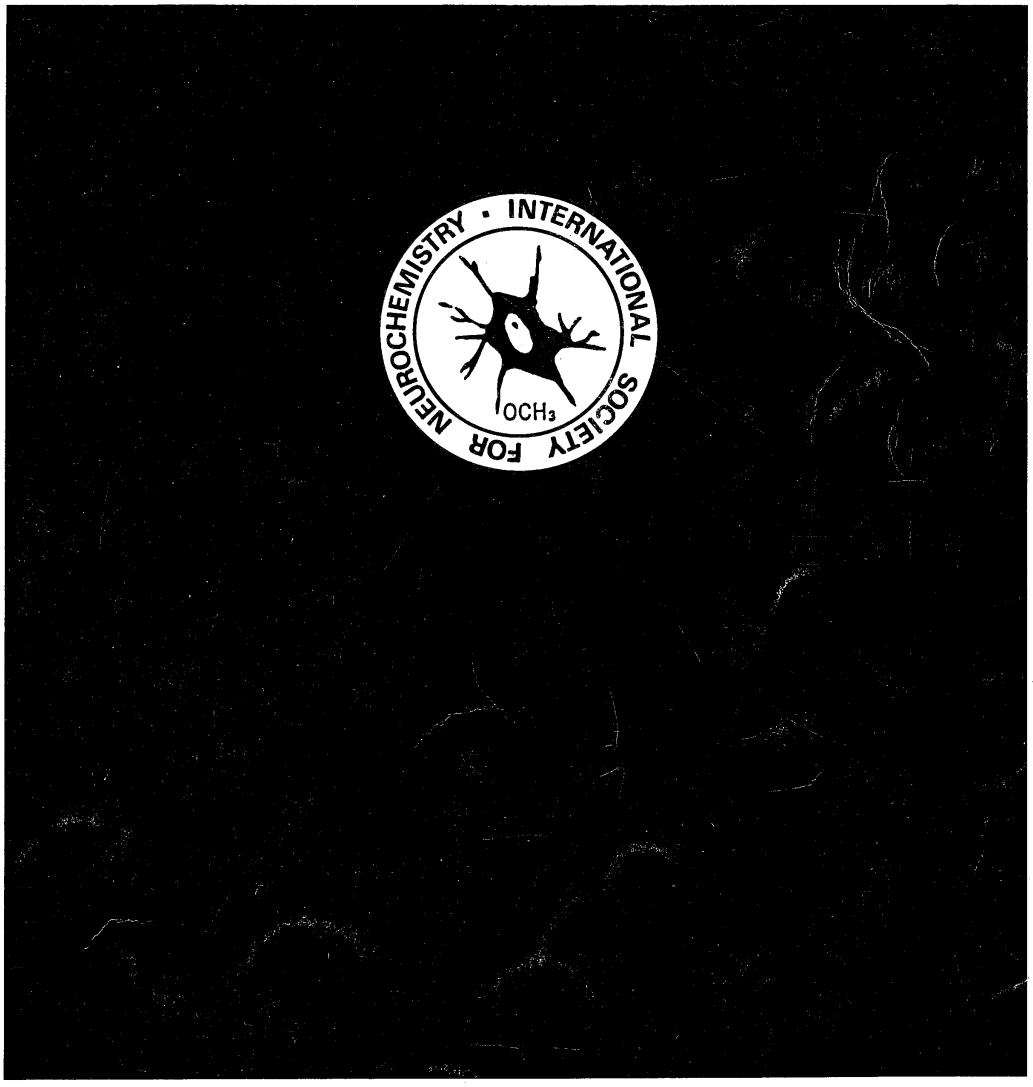

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Short Communication

Effects of Anticonvulsants on the Formation of γ-Hydroxybutyrate from γ-Aminobutyrate in Rat Brain

Susan R. Whittle and Anthony J. Turner

Department of Biochemistry, University of Leeds, Leeds, U.K.

Abstract: The conversion of γ-aminobutyrate (GABA) via succinic semialdehyde to γ-hydroxybutyrate has been examined in rat brain homogenates. A number of anticonvulsants, including sodium valproate and phenobarbitone, inhibited this metabolic pathway. These results are interpreted in the light of the characteristics of aldehyde reductases known to reduce succinic semialdehyde. **Key Words:** Aldehyde reductase—Anticonvulsants—Sodium valproate—γ-Hydroxybutyrate—GABA metabolism—Succinic semialdehyde. **Whittle S. R. and Turner A. J.** Effects of anticonvulsants on the formation of γ-hydroxybutyrate from γ-aminobutyrate in rat brain. *J. Neurochem.* **38,** 848–851 (1982).

Much attention has focussed on a role for γ-aminobutyrate (GABA) in the control of epileptic convulsions (Meldrum, 1975; Turner and Whittle, 1980). A disruption of metabolism that causes a fall in the synaptic concentration of GABA could lead to a decrease in neuronal inhibition resulting in seizures. Alternatively, the concentration of GABA may be normal but its postsynaptic action could be impaired. Anticonvulsants might restore the inhibitory response to normal by acting at either of these two levels. It has been known for some years that the anticonvulsant sodium valproate (Epilim®, 2-propylpentanoate) inhibits the conversion of GABA to its major metabolite, succinate (Harvey et al., 1975; Sawaya et al., 1975). However, it appears unlikely that this inhibition would be effective at normal clinical doses of the drug (Whittle and Turner, 1978; Turner and Whittle, 1980; Kerwin and Taberner, 1981). An alternative route of metabolism of GABA involves its transamination to succinic semialdehyde followed by reduction to γ-hydroxybutyrate. This compound is apparently capable of inducing an epileptic-like state in animals (Marcus et al., 1967; Snead et al., 1976). Thus alterations in brain levels of γ-hydroxybutyrate may affect the seizure threshold and are a potential site of action for valproate and similar compounds (Turner and Whittle, 1980; Kerwin and Taberner, 1981).

The enzyme(s) responsible for the reduction of succinic semialdehyde to γ-hydroxybutyrate may be related to the family of NADPH-dependent aldehyde reductases (alcohol:$NADP^+$ oxidoreductase, EC 1.1.1.2) (Kaufman et al., 1979). The major form of this enzyme has been shown to be very sensitive to inhibition by valproate, barbiturates, and some other anticonvulsants (Erwin and Dietrich, 1973; Whittle and Turner, 1978; 1981*a,b;* Javors and Erwin, 1980). Recently, however, a distinct reductase exhibiting high specificity and affinity for succinic semialdehyde has been reported to occur in brain (Cash et al., 1979; Hoffmann et al., 1980; Rumigny et al., 1980; Rivett et al., 1981). This latter enzyme is apparently insensitive to inhibition by anticonvulsants (Cash et al., 1979; Rumigny et al., 1980). The relative contribution of each of these reductases to the physiological production of γ-hydroxybutyrate from GABA has not been directly assessed. Clarification of the problem should reveal whether modifications in this metabolic pathway are relevant to the molecular actions of certain classes of anticonvulsants, as suggested elsewhere.

The concentration of succinic semialdehyde in brain is normally very low (approximately 10^{-10} mol/g brain—Matsuda and Hoshino, 1977). Several authors have therefore argued that the specific and 'high-affinity' succinic semialdehyde reductase would be the principal, if not exclusive, enzyme involved in γ-hydroxybutyrate formation from GABA (Hoffmann et al., 1980; Rumigny et

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Address correspondence and reprint requests to Dr. A. J. Turner, Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.

Abbreviation used: GABA, γ-Aminobutyric acid.

al., 1981; Rivett et al., 1981). However, Anderson et al. (1977) have shown that barbiturates markedly inhibit γ-hydroxybutyrate formation *in vitro*, suggesting a significant role for the major aldehyde reductase in this process. These authors, though, used millimolar concentrations of succinic semialdehyde as substrate. To obtain a more realistic assessment of the rates of synthesis of γ-hydroxybutyrate, we examined the effects of anticonvulsants, valproate analogues, and other known aldehyde reductase inhibitors on the overall formation of γ-hydroxybutyrate from GABA in rat brain homogenates. Studies of this type have previously allowed us to assess the relative contribution of aldehyde reductase isoenzymes to the reductive metabolism of catecholamines (Whittle and Turner, 1981*a*).

MATERIALS AND METHODS

Sodium valproate was a gift from Dr. D. S. Walter, Reckitt & Colman Pharmaceutical Division, Hull. The flavonoid inhibitors quercetin and quercitrin were obtained respectively from Sigma Chemical Corp. and from I.C.N. Pharmaceuticals Inc., Piscataway, New Jersey, U.S.A. γ-Valerolactone was from Aldrich Chemical Co., Gillingham, U.K. GABA aminotransferase (4-aminobutyrate:2-oxoglutarate aminotransferase, EC 2.6.1.19) was purified from rat brain and assayed as described previously (Whittle and Turner, 1978).

Brains obtained from male Wistar rats (170–190 g) were homogenised in 1 volume of 0.1 M-sodium phosphate buffer, pH 7.0, and a sample (equivalent to 10 mg brain) was incubated at 37°C for 5 min in the presence of GABA (1 mM), 2-oxoglutarate (1 mM) and NADPH (0.1 mM). The reaction was terminated by the addition of 2 ml of trichloroacetic acid (50%, w/v). Denatured protein was removed by centrifugation and the supernatant was lyophilised. The residue was redissolved in 0.1 M-sodium phosphate buffer, pH 7 (1 ml), and heated at 90°C for 10 min to convert the acid to a lactone derivative. After cooling on ice, the sample was adjusted to pH 6 and γ-valerolactone (2 μg) was added as internal standard. The sample was then extracted twice with 2 volumes of chloroform, and the pooled chloroform extracts were evaporated to approximately 100 μl before samples were quantitated by gas-liquid chromatography. The production of γ-hydroxybutyrate was linear with respect to time and protein concentration in the range tested. The recovery of product, determined by adding a known amount of γ-hydroxybutyrate to the brain homogenate followed by the normal extraction procedure, was 85 ± 5%.

RESULTS

The uninhibited reaction produced 0.107 ± 0.018 μg γ-hydroxybutyrate/min/g brain (wet weight) in the presence of NADPH. The reaction rate in the presence of added coenzyme was threefold that observed in its absence. NADH, however, produced no significant stimulation of reaction rate suggesting that the formation of γ-hydroxybutyrate from GABA is primarily NADPH-dependent. These data would be consistent with the report by Cash et al. (1979) that brain aldehyde reductases using succinic semialdehyde as substrate are NADPH-dependent.

The effects of a range of anticonvulsants and aldehyde reductase inhibitors on γ-hydroxybutyrate formation are listed in Table 1. Sodium valproate and its structural analogues, as well as phenobarbitone, all inhibited the reaction. The aldehyde reductase inhibitors quercetin and quercitrin also exerted significant inhibitory effects. The overall conversion of GABA to γ-hydroxybutyrate involves the action of GABA aminotransferase (EC 2.6.1.19) as well as aldehyde reductases. Thus, the effects of those compounds that inhibited γ-hydroxybutyrate

TABLE 1. *Effects of anticonvulsants and valproate analogues on γ-hydroxybutyrate formation from GABA*

Drug (1 mM)	γ-Hydroxybutyrate formed (μg/min/g brain)	Inhibition (%)
None	0.107 ± 0.018 (9)	—
Anticonvulsants		
Sodium valproate	0.035 ± 0.002 (4)	67.3
Phenobarbitone	0.040 ± 0.008 (4)	62.6
Diphenylhydantoin	0.088 ± 0.021 (4)	17.8
Carbamazepine	0.095 ± 0.01 (4)	11.3
Phenacemide	0.080 ± 0.011 (4)	25.2
Valproate analogues		
Diphenylacetate	0.038 ± 0.003 (4)	64.5
2-Phenylbutyrate	0.041 ± 0.003 (4)	61.7
2-Ethylhexanoate	0.042 ± 0.003 (4)	60.7
Other aldehyde reductase inhibitors		
Quercetin	0.029 ± 0.009 (4)	72.9
Quercitrin	0.048 ± 0.014 (4)	55.1

Samples (1 μl) obtained as described in the text were injected into a Varian Aerograph 1520 gas-liquid chromatograph containing a glass column (2 m × 3 mm i.d.) packed with 6% diethyleneglycol-succinate on 100–120 mesh Phasesep NI DCMS, and connected to a flame-ionization detector. The temperature settings were 167°C, 128°C and 150°C for inlet, column and detector, respectively, and carrier gas (helium) flow rate was 35 ml/min. Retention times were 2.6 min for γ-valerolactone and 1.7 min for γ-hydroxybutyrate.

Results are means ± S.D. for the numbers of animals indicated in parentheses.

TABLE 2. *Effects of inhibitors of γ-hydroxybutyrate formation on the activity of GABA aminotransferase*

Inhibitor	Rate (nmol/min/ml)	Inhibition (%)
None	6.11	—
Sodium valproate	5.6	8.4
Phenobarbitone	5.88	3.8
Diphenylacetate	5.95	2.6
2-Phenylbutyrate	5.63	7.9
2-Ethylhexanoate	5.80	5.1
Quercetin	5.95	2.1
Quercitrin	5.3	13.2

Rat brain GABA aminotransferase was purified and assayed as described for the enzyme from ox brain (Whittle and Turner, 1978). All inhibitors were maintained at 1 mM.

formation were also examined on the activity of the transaminase. None of the observed inhibitors of γ-hydroxybutyrate formation had a significant effect on the activity of purified GABA aminotransferase at the concentrations used in these studies (Table 2).

DISCUSSION

The present results suggest that the specific succinic semialdehyde reductase is not exclusively responsible for γ-hydroxybutyrate formation from GABA under the conditions used here, since this enzyme is reported to be unaffected by barbiturates and sodium valproate (Cash et al., 1979; Rumigny et al., 1980). It would appear that the nonspecific (anticonvulsant-sensitive) aldehyde reductase may also contribute to a significant extent to this metabolic pathway. These conclusions would not be inconsistent with the established data on the two reductases purified from rat brain. If it is assumed that the intracellular (noninulin) space in brain is 0.56 ml/g tissue (see e.g. Turner and Hick, 1975), then a value of approximately 1.8×10^{-7} M is obtained for normal intracellular concentrations of succinic semialdehyde (Matsuda and Hoshino, 1977). The specific reductase contributes 10–20% toward the total reductase activity in brain and both enzymes are cytosolic in location (Rumigny et al., 1980; 1981). The respective Michaelis constants for succinic semialdehyde are reported to be 28 μM (specific reductase) and 140 μM (nonspecific reductase), obtained with the purified enzymes from rat brain (Rumigny et al., 1980). Given these parameters, the nonspecific reductase would be expected to contribute more than 50% of the total activity toward succinic semialdehyde reduction at physiological concentrations of this substrate. Even at substantially lower concentrations of succinic semialdehyde, the nonspecific reductase would still make a significant contribution towards γ-hydroxybutyrate production, in agreement with our data (Table 1). We cannot at present, though, rule out the possibility that the specific succinic semialdehyde reductase may be localized in GABA-containing neurons.

Relatively high concentrations of sodium valproate compared with those in clinical use (Sawaya et al., 1975) are required to cause significant inhibition of γ-hydroxybutyrate formation (Table 1). Thus, it seems unlikely that valproate exerts its anticonvulsant effects through an action on this metabolic pathway. An alternative mode of action of valproate must therefore be postulated. An effect of valproate on the postsynaptic membrane is an attractive proposition in view of recent neurophysiological studies suggesting that valproate augments the postsynaptic inhibitory effects of GABA (MacDonald and Bergey, 1979; Kerwin et al., 1980).

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