MAO B and whether steric or electronic factors are of greater importance. Thus the real value of dinitranyl in structure/activity studies of propynylamines is that it perhaps points to the features which preclude binding to MAO B and hence confer specificity for MAO A. Studies by Knoll *et al.* [21] and more recently by Kalir *et al.* [22] have produced some structural criteria for MAO B selectivity in propynylamines, but so far the compounds do not seem to show a better selectivity than deprenyl.

In summary, a new propynylamine has been tested as an inhibitor of MAO. Loosely based upon the structure of clorgyline, it is an irreversible inhibitor of MAO A but is apparently indifferent towards MAO B. This compound, N^1-(2,4-dinitrophenyl)-N^2-prop-2-ynyl 1,3-diaminopropane, may be useful as a model for the design of more potent but equally specific inhibitors of MAO A.

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REFERENCES

1. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
2. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 393 (1972).
3. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **23**, 629 (1974).
4. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **24**, 1889 (1975).
5. C. H. Williams, *Ir. J. med. Sci.* **146**, 91 (1977).
6. C. H. Williams and F. Dollie, *Ir. J. med. Sci.* **147**, 71 (1978).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. T. P. Singer, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 7. Academic Press, New York (1979).
9. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19**, 467 (1976).
10. R. W. von Korff, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 1. Academic Press, New York (1979).
11. K. F. Tipton and L. Della Corte, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 87. Academic Press, New York (1979).
12. R. McAuley and E. Racker, *Molec. cell. Biochem.* **1**, 73 (1973).
13. R. M. Cawthon, J. E. Pintar, F. P. Haseltine and X. O. Breakefield, *J. Neurochem.* **37**, 363 (1981).
14. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24**, 2039 (1975).
15. I. S. Severina, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 169. Academic Press, New York (1979).
16. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135**, 173 (1973).
17. S. M. Russell, J. Davey and R. J. Mayer, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 265. Academic Press, New York (1979).
18. M. Sandler, V. Glover, J. D. Elsworth, R. Lewisohn and M. A. Reveley, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 447. Academic Press, New York (1979).
19. M. B. H. Youdim, P. Riederer, W. Birkmayer and J. Mendelwicz, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 477. Academic Press, New York (1979).
20. K. F. Tipton and T. J. Mantle, in *Monoamine Oxidase Inhibitors—The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 3. John Wiley, London (1981).
21. J. Knoll, Z. Eczery, K. Magyar and E. Satory, *Biochem. Pharmac.* **27**, 1739 (1978).
22. A. Kalir, A. Sabbagh and M. B. H. Youdim, *Br. J. Pharmac.* **73**, 55 (1981).

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Effects of anticonvulsants on aldehyde reductase and acyl-CoA reductase: implications for the biosynthesis of ether-linked glycerolipids in brain

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Plasmalogens (alk-1-enyl glycerolipids), which are particularly abundant in the membranes of nerve and muscle cells, are formed by desaturation of alkyl glycerolipids [1]. Metabolic and structural studies have established that the alkyl chains of these ether-linked glycerolipids are derived from long-chain aliphatic alcohols [2]. Their enzymic synthesis in brain is maximal during the period of active myelination [3].

Long-chain aliphatic alcohols appear to be synthesized by reduction of the corresponding fatty acids [2,4–6]. The overall reaction, which predominantly occurs in the microsomal fraction of nerve cells, may be dissected into two discrete steps. The first stage requires ATP, Mg^{2+} and coenzyme A and leads to the formation of an aliphatic aldehyde which, in the second step, is converted to an alcohol by NADPH-dependent aldehyde reductase [6]. However, the intermediate formation of the aldehyde in microsomal preparations has not been conclusively shown and the nature of the reductase has not been established.

A suggested candidate [1, 4, 6] for reduction of long-chain aliphatic aldehydes is the non-specific aldehyde reductase (alcohol:$NADP^+$ oxidoreductase, EC 1.1.1.2) present in many mammalian tissues which can reduce a variety of aromatic and aliphatic aldehydes [7–10]. This enzyme is cytosolic in location [8] and is sensitive to inhibition by anticonvulsant drugs including sodium valproate

(2-propylpentanoate) and phenobarbitone [11–13]. Its physiological function is unclear. In the present study we have taken advantage of the inhibitor sensitivity of this aldehyde reductase to examine whether it plays any significant role in glycerolipid biosynthesis in rat brain as previously suggested [1, 4, 6]. Evidence is additionally presented for the existence of a microsomal reductase that is relatively insensitive to inhibition by anticonvulsants.

Materials and methods. Aldehyde reductase activity was assayed spectrophotometrically at pH 7.2 as described previously [12]. Aldehyde reductase was purified from rat (150–200 g) brain cytosol by ammonium sulphate fractionation and DEAE–cellulose chromatography [14].

Subcellular fractionation of brain tissue was carried out as reported previously [5]. The microsomal pellet was washed by resuspension in 10 mM sodium phosphate buffer, pH 7.4, and centrifuged at 100,000 g_{av} for 30 min. The pellet was washed twice more and finally resuspended in 0.1 M phosphate buffer, pH 7.4. Aldehyde reductase was solubilised from the microsomal preparation by incubation for 30 min at 4° in the presence of 1% (w/v) Triton X-100. When subjected to centrifugation at 100,000 g_{av} for 30 min, more than 95% of the microsomal aldehyde reductase activity was in a soluble form and appeared in the supernatant solution. Incubation and centrifugation of the microsomal pellet in the presence of 0.2 M sodium phosphate buffer, pH 7.4 (in the absence of Triton) released no detectable activity into the supernatant. Freeze-thawing of microsomal preparations also failed to solubilize the enzyme suggesting that it was not occluded activity but due to the presence of an intrinsic membrane protein.

The conversion of palmitate to hexadecanol was measured as described elsewhere [6]. The assay system contained [1-^{14}C]palmitate (40 nmole, sp. act. 49,000 dpm/nmole) taken up in 10 μmole phosphate buffer pH 7.4, 8 μmole ATP, 8 μmole $MgCl_2$, 0.1 μmole coenzyme A, 1 μmole NADPH and microsomal protein (1.55 mg) in a total vol. 0.4 ml. The mixture was incubated at 37° for 1 hr. Lipids were then extracted and chromatographed on silica gel G using dichloroethane as solvent system and with unlabelled hexadecanol as standard. The area corresponding to hexadecanol was scraped from the plate and its radioactivity was determined. The amount of fatty alcohol formed was estimated from the specific activity of the precursor fatty acid used [6] and the enzyme activity was expressed as nmole/hr/mg protein.

Results and discussion. Sodium valproate and phenobarbitone had no significant effect on hexadecanol formation in the concentration range 0.1–5 mM (Table 1). However, the major aldehyde reductase purified from rat brain was almost completely inhibited by both drugs (Table 2) suggesting that it was not involved in the biosynthesis of hexadecanol.

The washed microsomal fraction from rat brain exhibited NADPH-dependent aldehyde reductase activity (Table 2). This activity constituted only 4–6% of the total aldehyde reductase activity present in the original homogenate. The microsomal reductase could be solubilised from the membrane preparation by detergent treatment. The enzyme, in both membrane-bound and solubilised forms, was relatively insensitive to inhibition by sodium valproate and phenobarbitone (Table 2) and could therefore be distinguished from the major reductase activity in brain [12]. This microsomal aldehyde reductase, which could also reduce dodecylaldehyde and palmitaldehyde *in vitro*, may participate in hexadecanol formation in microsomal prep-

Table 1. Effects of sodium valproate and phenobarbitone on the NADPH-dependent conversion of palmitate to hexadecanol

Incubation conditions	Drug concentration (mM)	[^{14}]Hexadecanol formed (nmole/hr/mg protein)
Control	—	1.31
+Sodium valproate	0.1	1.19
	0.5	1.32
	1.0	1.34
	5.0	1.45
+Phenobarbitone	0.1	1.05
	0.5	1.22
	1.0	1.37
	5.0	1.17

The formation of [^{14}C]hexadecanol from [1-^{14}C]palmitate by brain microsomes was measured as described in the text. Drugs were added as aqueous solutions to give the final concentrations shown below. The brains of 10-day-old rats were used.

Table 2. Effects of anticonvulsants on microsomal and cytosolic NADPH-dependent aldehyde reductases

Drug	Drug concentration (mM)	Activity (%) Cytosolic reductase	Microsomal reductase
Sodium valproate	0.1	38 ± 5	96 ± 8
	1.0	<5	91 ± 6
Phenobarbitone	0.1	42 ± 4	98 ± 5
	1.0	<5	93 ± 6

The major aldehyde reductase activity in rat brain (cytosolic form) was purified as described in the text before being assayed as described previously [12]. The microsomal aldehyde reductase was solubilised from the membrane with detergent before assay. Triton X-100 (1%, w/v) did not significantly affect the activity of either enzyme in the presence or absence of inhibitors. The results are the mean ± S.D. of 4 separate enzyme preparations.

arations from brain. The development of a specific inhibitor of this reductase will, however, be required to test this hypothesis.

The sensitivity of the major aldehyde reductase in brain to a wide range of anticonvulsants has led to the suggestion that modifications in the levels of aldehydes might explain, in part, the actions of drugs used in the control of seizures [11, 13, 15]. Although modifications in membrane lipid metabolism would be an attractive site of action for anticonvulsants, our data failed to demonstrate any significant action of these drugs on the formation of long-chain aliphatic alcohols. The identification of a distinct microsomal aldehyde reductase further argues against a significant role of the valproate-sensitive enzyme in the biosynthetic pathway to ether-linked glycerolipids. The physiological function of this abundant reductase in brain therefore remains unresolved.

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REFERENCES

1. R. L. Wykle and F. Snyder, in *The Enzymes of Biological Membranes* (Ed. A. Martonosi), Vol. 2, pp. 87–117. Wiley, London (1976).
2. F. Snyder, B. Malone and M. L. Blank, *J. biol. Chem.* **245**, 1790 (1970).
3. F. Snyder, M. Hibbs and B. Malone, *Biochim. biophys. Acta* **231**, 409 (1971).
4. J. C. Kawalek and J. R. Gilbertson, *Archs Biochem. Biophys.* **173**, 649 (1976).
5. V. Natarajan and P. S. Sastry, *J. Neurochem.* **23**, 187 (1974).
6. V. Natarajan and P. S. Sastry, *J. Neurochem.* **26**, 107 (1976).
7. B. Tabakoff and V. G. Erwin, *J. biol. Chem.* **245**, 3262 (1970).
8. A. J. Turner and K. F. Tipton, *Eur. J. Biochem.* **30**, 361 (1972).
9. T. G. Flynn, J. Shires and D. J. Walton, *J. biol. Chem.* **250**, 2933 (1975).
10. A. J. Turner and J. Hryszko, *Biochim. biophys. Acta* **613**, 256 (1980).
11. V. G. Erwin and R. A. Deitrich, *Biochem. Pharmac.* **22**, 2615 (1973).
12. S. R. Whittle and A. J. Turner, *J. Neurochem.* **31**, 1452 (1978).
13. M. Javors and V. G. Erwin, *Biochem. Pharmac.* **29**, 1703 (1980).
14. S. R. Whittle and A. J. Turner, *Biochim. biophys. Acta* **657**, 94 (1981).
15. M. M. Ris, R. A. Deitrich and J. P. von Wartburg, *Biochem. Pharmac.* **24**, 1865 (1975).

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