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Letter to the Editor

Antiepileptic activity of 1,3-dihexadecanoylamino-2-valproyl-propan-2-ol, a prodrug of valproic acid endowed with a tropism for the central nervous system

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The potential of diacylglycerols as vectors of carboxylic acids, designed to facilitate their passage through the intestinal (Garzon-Aburbeh et al 1983, 1986) and haematoencephalic barriers (Jacob et al 1985, 1987, 1990) has received considerable attention in recent years. Although the clinical usefulness of valproic acid as an antiepileptic drug is well-established (Bruni & Wilder 1979; Chapman et al 1982), this compound suffers two major drawbacks: an early prominent plasma concentration peak (Gugler & von Unruh 1980) and various adverse side-effects such as nausea, somnolence, tremor, decreased platelet function and hepatotoxicity (Haas & Bourgeois 1989). Some of these adverse effects may be related to this sharp concentration peak. Thus, a perturbation of the mitochondrial function has been evidenced at similar concentration levels (Draye & Vamecq 1987). This problem may perhaps be overcome by designing prodrugs which would regulate the plasma concentrations and, optimally, would be endowed with a tropism for the central nervous system (CNS). In this connection and in view of the success met with diacylglycerol-GABA adducts (Jacob et al 1985, 1987, 1990), we have explored the antiepileptic activity of similar prodrugs of valproic acid (1–3) (Fig. 1) with special emphasis on the determination of the time of maximal intensity of anticonvulsant action.

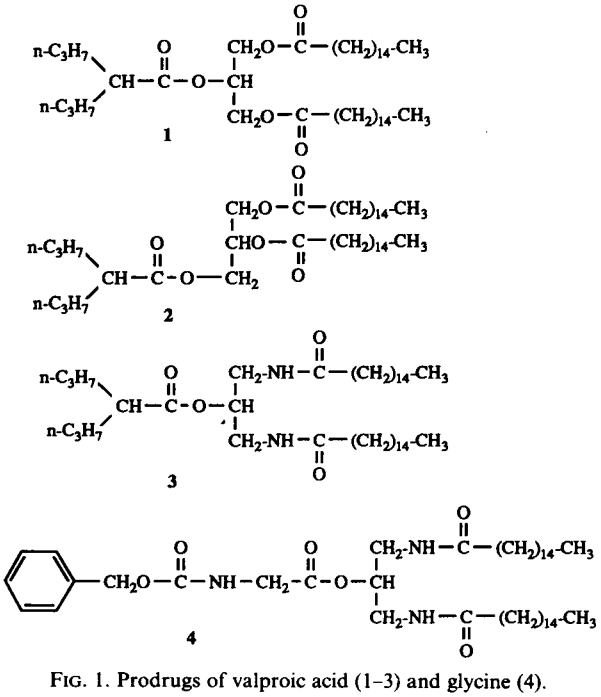

FIG. 1. Prodrugs of valproic acid (1–3) and glycine (4).

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Compounds 1, 2 were synthesized in two steps with yields of 68–79%. First, *O,O'*-1,3-diacylpropan-1,2,3-triols were obtained according to the method of Bentley & McCrae (1970), using the corresponding acyl chlorides. A similar procedure allowed us to obtain the *N,N'*-dipalmitoyl-1,3-diaminopropan-2-ol molecule necessary for 3–4 (Mergen et al 1991). Subsequently, target compounds 1–3 were synthesized by interaction at room temperature (21°C) of the 1,3-diglycerides with 2-propyl-pentanoyl chloride in anhydrous THF in the presence of triethylamine and catalytic amounts of 4-dimethylaminopyridine (Höfle et al 1978). The final compounds were analytically pure (within 0·4%), chromatographically homogenous (TLC, Silica Gel $60F_{254}$, chloroform:acetone, 90:10 v/v; HPLC, Zorbax CN, 25 × 0·46 cm, n-hexane:propan-2-ol, 95:5 v/v)) and possessed spectral properties (^{13}C-NMR, FAB-MS) consistent with their structures. Melting points were 41–42, 35–36 and 96–98°C, respectively.

Two well-standardized tests, the maximal electroshock seizure (MES) and the subcutaneous metrazol seizure threshold (scMet) tests were performed according to the protocol of Krall et al (1978). Tests were performed 15, 30, 60, 150 and 240 min after oral administration of the compounds to male NMRI mice, 20–25 g. Compounds 1, 2 were found inactive in the two tests at doses up to 1 mmol kg^{-1} p.o., while 3 showed activity in the scMet test (ED50 0·760 (0·600–0·925) mmol kg^{-1} p.o., t = 150 min). Compounds 1, 2 were also screened for anticonvulsant activity in mice (phase I) by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, of the National Institute of Neurological and Communicative Disorders and Stroke. They were found inactive in the MES, scMet and rotorod tests at doses up to 300 mg kg^{-1}. Gas–liquid chromatography indicated total disappearance from plasma of the 1 and 2 esters 10 min after i.v. injection. This is consistent with the observation made by Goncalves Saraiva (1990) that the substitution of a fatty acid chain by a xenobiotic moeity does not significantly modify the triglyceride's plasma half-life as compared with endogenous physiological triglycerides. Thus, the lack of activity of compounds 1 and 2 can be ascribed, at least partially, to their instability in the plasma. These results may confirm the absence of activity of glyceryl trivalproate and related ester prodrugs of valproate (Salim et al 1990). Interestingly, compound 3 was far more active than valproic acid (scMet-ED50 2·58 (2·32–2·94) mmol kg^{-1} p.o. t = 30 min) and its peak of action was considerably delayed. Moreover, the finding that the scMet-ED50 of 3 after oral administration was in the same range as that measured after i.p. injection (ED50 0·98 (0·80–1·10) mmol kg^{-1}, t = 15 min) denotes a good bioavailability of this compound.

This does not fully explain the divergent character of compound 3 compared with 1 and 2. Does the 1,3-diaminopropyl moiety confer a peculiar tropism for the CNS? To answer this question, we designed compound 4, a glycine prodrug (Fig. 1). Compound 4 was synthesized by stirring overnight, at room temperature, equivalent amounts of 1,3-dipalmitoylamino-propan-2-ol, (*N*-Cbz)-glycine and isopropenyl chloroformate

(Jouin et al 1987) in the presence of freshly distilled triethylamine and 4-dimethylaminopyridine in solution in anhydrous THF. After purification by column chromatography (silica gel, dichloromethane:acetone, 3:1 v/v), 4 was analytically pure and was characterized by ^{13}C-NMR; melting point 82–83°C.

It should be stressed that glycine cannot cross the blood–brain barrier because this amino acid does not possess any specific carrier (Aprison & Daly 1978). Moreover, a glycine receptor is coupled to the acidic amino acid receptor complex and glycine potentiates the action of GABA (Krogsgaard-Larsen et al 1988). Glycine may therefore play a role in the regulation of the electrical cortical activity. Compound 4 was indeed found active in the MES test (ED50 0·139 (0·084–0·229) mmol kg^{-1} i.p., t = 30 min). Neither *N*-benzyloxycarbonylglycine, nor 1,3-diaminopalmitoyl-propan-2-ol demonstrated significant protection against electroshock-induced seizures at similar dose levels. At present, as no detailed metabolic studies are available on compounds 3 and 4, it cannot be decided conclusively by which mechanism, either as such or as prodrugs, these compounds may act. Kohn recently reported anti-MES activity for *N*-acetyl-D,L-phenylglycine-*N*-benzylamide (Kohn et al 1988, 1990). It cannot be excluded, therefore, that compounds 3 and 4 might act as such. The long delay, however, after which compound 3 shows activity favours the prodrug hypothesis.

In conclusion, these first results indicate that replacement of the ester functions in 1,3-dipalmitoylglycerol by amide groups produces an orally active prodrug of valproic acid endowed with a tropism for the CNS. We are currently exploring additional variations of compounds 3 and 4 to delineate the optimal structure of the fatty acid side-chains as well as to enhance our understanding of the exact nature of their mechanism of action.

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