Effects of Ethyl Ester Derivatives of Valproic Acid Metabolites on Pentylenetetrazol Seizures in Mice

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SUMMARY: *The anticonvulsant activity of the ethyl esters of the major valproic acid metabolites was assessed against minimal pentylenetetrazol seizures in adult male ICR mice. The ethyl ester 3-hydroxy-propylpentanoic acid was found to possess significant anticonvulsant activity.*

RÉSUMÉ: *Nous avons étudié l'activité anticonvulsivante des esters ethylés des principaux métabolites acides de l'acide valproique. Cette activité fut évaluée sur les convulsions minimales au pentylenetetrazol chez la souris ICR mâle adulte. L'ester éthylé de l'acide 3-Hydroxy-propylpentanoique a démontré une activité anticonvulsivante significative.*

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INTRODUCTION

The anticonvulsant properties of commonly used antiepileptic drugs may be solely due to the administered parent compound (eg. phenytoin, phenobarbital, ethosuximide) or may be partly or entirely due to active metabolites (as is the case for primidone, carbamazepine, diazepam, methsuximide). Valproic acid is a broad spectrum anticonvulsant drug which is most effective in the prevention of absence and myoclonic seizures but also effective in other seizure types. The classic model for screening drugs to determine their efficacy in the prevention of clinical absence seizures is the prevention of pentylentetrazol-induced minimal threshold seizures in mice or rats (Swinyard, 1969). In these experiments we studied the anticonvulsant properties of the ethyl esters of the major valproic acid metabolites in this animal model.

MATERIALS AND METHODS

Adult male ICR mice weighing 25 to 45 gm were used as experimental animals. All animals were subjected to similar day-night cycles, were housed in laboratory cages, and were allowed free access to food and water except during the time of testing. The subcutaneous pentylenetetrazol seizure threshold test was employed with a pentylenetetrazol dose of 85 mg/kg (0.85% solution in 0.9% sodium chloride) injected subcutaneously into a skin fold on the dorsal aspect of the neck. The pentylenetetrazol solution was freshly prepared for each experiment. The intraperitoneal route was used for the administration of the metabolites. All test drugs were dissolved in propylene glycol to make desired drug concentrations in a small volume of injection which ranged from 0.06 to 0.09 cc in order to prevent the volume of solution from contributing significantly to total body water. At the time of testing, the identity of the compounds was unknown to the investigators and only at the completion of all the experiments were the compounds identified.

The test compounds were injected in four test doses of 120, 240, 360, and 480 mg/kg at 10, 20, and 30 minutes prior to the injection of pentylenetetrazol. After preliminary testing of the propylene glycol vehicle alone, groups of 5 animals were employed for each experiment. Following pentylenetetrazol administration, each animal was observed separately for 30 minutes by two observers who looked for seizure activity. Protection was defined as the absence of a single 5 second episode of clonic spasm (Swinyard, 1972). If protection was suggested, 1 or 2 additional groups of animals were tested under the same conditions of dosage and time prior to the administration of pentylenetetrazol.

Specific toxicity studies were not performed. However, observations were made with regard to the development of lethargy and ataxia while the animals moved freely in observation cages.

The compounds (supplied by Abbott Laboratories) were later identified as the ethyl ester of 3-keto-propylpentanoic acid (3 ketoPPA) (compound 1), the ethyl ester of valproic acid (compound 2), and the ethyl ester of 3-hydroxy-propylpentanoic acid (3 OH PPA) (compound 3). The metabolites of valproic acid are unstable and the ethyl esters were synthesized to impart stability.

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RESULTS

Pre-treatment with compounds 1, 2 and 3 in doses of 120, 240, and 360 mg/kg failed to protect any animals when administered 10, 20 and 30 minutes prior to the administration of pentylenetetrazol. No toxicity was noted and the seizures were similar to those observed in the controls. Compounds 1 and 2 were also ineffective when administered in doses of 480 mg/kg at the three time intervals. Only compound 3 (the ethyl ester of 3 OH PPA) was found to offer protection (Figure 1).

TEST SUBSTANCE	TIME PRIOR TO PTZ ADMINISTRATION (MINUTES)		
	10	20	30
Controls	0	0	0
Compound 1	0	0	0
Compound 2	0	0	0
Compound 3	66	30	0

Figure 1: Percent of animals protected with 480 mg/kg dose of test compounds.

The ethyl ester of 3 OH PPA protected 66% of the animals (10/15) when administered 10 minutes prior to pentylenetetrazol and 30% of the animals (3/10) when administered 20 minutes prior to pentylenetetrazol (see Figure 1). Statistical significance was only reached with the former time schedule ($p < 0.05$, Chi Square test). Isolated clonic body jerks were observed between 15 and 30 minutes in the majority of the protected animals. Only with this compound was lethargy and ataxia observed. The animals began to develop sedation and ataxia at 2 minutes. These remained maximal until approximately 10 to 12 minutes but moderate sedation and ataxia were still obvious at 30 minutes. The time course of protection paralleled signs of toxicity.

DISCUSSION

Valproic acid, the most recently introduced major antiepileptic drug, was first discovered to possess anticonvulsant properties in 1963 (Meunier et al, 1963). Since that time extensive clinical use has shown that valproic acid is most effective in the prevention of absence and myoclonic seizures (Bruni and Wilder, 1979; Pinder et al, 1977; Simon and Penry, 1975). It has also been observed that a delay may be present before the therapeutic effects in humans are produced despite stable therapeutic plasma concentrations of valproic acid. There is no known explanation for this delay in both clinical and electroencephalographic effects (Bruni et al, 1980). This may be partly due to the time required for optimum brain concentration of valproic acid to be achieved. A second possibility is that an optimum brain concentration of metabolites may also be necessary. The time course of entry of these compounds into the central nervous system is presently unknown and no binding to brain structures occurs (Goldberg and Todoroff, 1980).

In spite of extensive clinical and pharmacological studies of valproic acid, its metabolism has been less clearly defined. One of the difficulties has been that species differences exist in its metabolism (Ferrandes and Eymard, 1977, Gompertz et al, 1977; Gugler et al, 1977; Jakobs and Loscher, 1978; Kuhara and Matsumoto, 1974; Matsumoto et al, 1976). In humans, it has recently been confirmed that both β - and ω - oxidation are involved in the metabolism of valproic acid (Gompertz et al, 1977; Jakobs and Loscher, 1978; Kuhara and Matsumoto, 1974). Three-hydroxy-propylpentanoic acid appears to be the major human metabolite found in serum and 3-keto-propylpentanoic acid appears to be the major human metabolite isolated from urine. Small quantities of 4-hydroxy-propylpentanoic acid are also present in serum.

In these experiments the ethyl esters of the naturally occurring metabolites were used. These ester forms do not occur in vivo and caution must be exercised in directly extrapolating our results to the naturally occurring metabolites. However our observations suggest that the addition of the ethyl ester reduces anticonvulsant activity since this derivative of valproic acid lacked anticonvulsant activity. If this is so, one may predict that the naturally occurring metabolites may have a greater anticonvulsant effect than the ethyl ester derivative. Whether esterification changes the ability of these compounds to enter the central nervous system has also to be considered. Another factor that has to be considered in vivo is the relative quantity of these metabolites and it is presently unknown whether the concentrations used in these experiments are reached in the clinical situation.

The ED_{50} of valproic acid in mice against pentylenetetrazol seizures is often quoted as 120-122 mg/kg (Shuto and Nishigaki, 1970) and we have found valproic acid to protect 80 percent of mice when administered in a 240 mg/kg dose at 10 minutes prior to pentylenetetrazol administration and 40 percent of animals when administered in a 120 mg/kg dose (Bruni, Hammond, Wilder, unpublished data, February, 1979). Therefore it would be of interest to compare the anticonvulsant properties of 3 OH PPA metabolite without esterification with those of valproic acid. Preliminary observations suggest that the total anticonvulsant effect of valproic acid may be partly related to some of its metabolites.

The further metabolism of 3 OH PPA to 3 keto PPA by β - oxidation results in a compound whose ethyl ester derivative lacks anticonvulsant properties. The naturally occurring 3 keto PPA however, may have anticonvulsant properties (Schafer et al, 1980).

The pharmacological activity of this major valproic acid metabolite also raises the possibility of long term toxicity being related to this compound rather than to the parent drug. This may be an important consideration in

the treatment of patients with a decreased ability to excrete the metabolite (eg. renal disease). It is hoped that human pharmacological studies will be carried out to determine the pharmacokinetic parameters of this active metabolite.

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