Brain Research, 457 (1988) 371–375
Elsevier

BRE 23028

Marked stereospecificity in a new class of anticonvulsants

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(Accepted 26 April 1988)

Key words: Anticonvulsant; Stereoselectivity; Maximal electric shock; Mouse

N-Acetyl-D,L-alanine-*N*-benzylamide and *N*-acetyl-D,L-phenylglycine-*N*-benzylamide are two novel anticonvulsants that selectively block maximal electric shock-induced tonic extensor seizures in mice. For both compounds, the anticonvulsant activity is due to the D-stereoisomer, and the L-stereoisomer is virtually inactive as an anticonvulsant. The marked stereoselectivity of these anticonvulsants may make them very useful pharmacological tools for the study of the mechanism(s) of anticonvulsants that selectively inhibit maximal electric shock-induced seizures.

The prototypical anticonvulsant drugs for the treatment of partial and generalized tonic-clonic seizures are phenobarbital, phenytoin and carbamazepine[14]. In mice and rats, these prototypical anticonvulsants effectively prevent maximal electric shock (MES)-induced seizures at doses lower than those which produce neurological impairment[18]. Recently, a novel series of anticonvulsants, the functionalized amino acid derivatives, has been discovered with a similar selectivity for MES-induced seizures[4,5]. Two of the most active compounds from this series are the *N*-acetyl-D,L-alanine-*N*-benzylamide (D,L-AAB) and the *N*-acetyl-D,L-phenylglycine-*N*-benzylamide (D,L-APB) (referred to as 1a and 1d, respectively, in ref. 4). The D,L-AAB had an ED_{50} of 76.54 mg/kg (i.p.) in mice against MES seizures, was inactive at 600 mg/kg (i.p.) against Metrazol-induced clonic seizures, and had an ED_{50} for producing neurological impairment of 453.86 mg/kg (i.p.). The D,L-APB was more potent, with an MES TD_{50} of 20.31 mg/kg (i.p.), was also inactive against Metrazol-induced clonic seizures, and produced an TD_{50} for neurological impairment of 96.92 mg/kg (i.p.). Thus, these two compounds had similar anticonvulsant profiles to phenytoin; that is, they were selective for MES seizures, compared to Metrazol-induced seizures, and had respectable P.I.'s (protective index = neurological impairment TD_{50} divided by MES ED_{50}).

A number of racemic anticonvulsant agents have been resolved and the anticonvulsant activities of the individual stereoisomers have been studied[1]. Typically, qualitatively similar anticonvulsant activities were seen with both stereoisomers, with only small differences in potencies. Nirvanol, the demethylated metabolite of mephenytoin, has been reported to display the largest difference in activity between its two stereoisomers. The (R)-stereoisomer was 3.8 times more potent than the (S)-stereoisomer against seizures induced by electroshock in mice[1]. Recently, the stereoisomers of a novel anticonvulsant, LY188544 (S,R-4-amino-*N*-(α-methylbenzyl)benzamide), have been studied for anticonvulsant activity[10]. After oral administration, the (S)-stereoisomer was 2.2 times more potent than the (R)-stereoisomer in the MES test. However, after i.v. administration the two stereoisomers were approximately equal in potency. The purpose of the present research was to evaluate the stereoisomers of D,L-AAB and D,L-APB to determine if there was isomeric selectivity within this series of novel anticonvulsants. All of the compounds used in this study were synthesized from the appropriate chiral or D,L-amino acid, using procedures

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common for the preparation of peptides[2]. The D- and L-enantiomers of AAB were prepared from the corresponding optically pure alanylmethyl ester hydrochlorides using the conditions employed for the racemate[4]. An alternative route was employed for the synthesis of the D- and L-enantiomers of APB. In this series, the appropriate chiral phenylglycine was converted to the *N*-*t*-butoxycarbonyl derivative and then coupled with benzylamine using the mixed anhydride method (ethyl chloroformate, triethylamine). Deprotection (trifluoroacetic acid), followed by acetylation (acetyl chloride, triethylamine) afforded the desired product. The racemates have been previously delineated[4]. The enantiomeric purity of the isomeric final products was supported by their observed optical rotations [for D-AAB, $[\alpha]^{23} = +36.2$ (2.5% in MeOH); L-AAB, $[\alpha]^{23} = -35.2$ (2.5% in MeOH); D-APB, $[\alpha]^{23} = -103.0$ (1% in EtOH); L-APB, $[\alpha]^{23} = +105.1$ (1% in EtOH)] as well as by NMR studies using the chiral shift reagent, (+)-2,2,2-trifluoro-1-(9-anthryl)ethanol[13].

The D- and L-stereoisomers of both compounds were evaluated for anticonvulsant activity using the MES test. Some of the data were generated by the Antiepileptic Drug Development (ADD) program of the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke[8,15], whereas the rest of the data were generated at Lilly Research Laboratories. Both laboratories used male albino mice (CF-1 strain, 18–25 g; Charles River Breeding Laboratories, Portage, MI) as experimental subjects. The mice were allowed free access to food and water, except when they were removed from their colony cages for the experimental procedures. For i.p. administration, the compounds were administered in 30% polyethylene glycol 400/water mixture in a volume of 0.01 ml/g b. wt.

The MES test measures the ability of the test drug to abolish the hind limb extensor component of maximal seizures induced by 50 mA of 60-cycle current delivered for 0.2 s via corneal electrodes. This amount of stimulation is approximately 6 times the threshold and reveals the ability of the compound to prevent seizure spread. To determine selectivity for anticonvulsant effects, the compounds were also studied for their effects to neurologically impair the mice. At the Epilepsy Branch, the neurologically impairing effect was studied using the rotorod procedure[7]. When a normal mouse is placed on a knurled rod 1 inch in diameter rotating at a speed of 6 rpm, it can maintain its equilibrium for at least 1 min. Inability to do this was defined as 'neurological impairment'. At Lilly Research Laboratories, the horizontal screen test was used to determine neurological impairment[6]. Previously trained mice were dosed with the compound and placed individually on top of a square (13 cm × 13 cm) wire screen (no. 4 mesh) which was mounted on a metal rod. The rod was rotated 180°, and the number of mice that returned to the top of the screen was determined. Inability to climb to the top within 1 min was defined as 'neurological impairment'.

Each compound was tested at various time intervals to determine the time of peak effect. The time of peak effect after i.p. administration for these compounds was 0.5 h and that is the time at which dose–response data were generated. From the dose–response curves (with at least 4 doses from no-effect to complete protection), the dose of compound which was estimated by computer probit analysis to protect 50% of the mice from MES-induced tonic extensor seizures was defined as the MES-ED_{50}. The dose estimated to produce neurological impairment in 50% of the mice was defined as the toxic $dose_{50}$ (TD_{50}).

The two racemates, AAB and APB, and the two stereoisomers of each were also studied on the MES test at 5 min after i.v. administration in an attempt to determine intrinsic activity without the potential problems of absorption, distribution and metabolism obscuring the pharmacological effects. For the i.v. study, the test compounds were administered in the tail vein in a solution of 5% emulphor, 5% ethanol and 90% water[3].

Table I shows the MES-ED_{50} and TD_{50} values for the various compounds. At the Epilepsy Branch, D,L-AAB had an MES-ED_{50} of 76.54 mg/kg. The D-stereoisomer was more potent, with an ED_{50} of 54.80 mg/kg, and the L-stereoisomer was 10-fold less potent than the D-isomer. D-AAB was also more potent than both the racemate and the L-stereoisomer in producing neurological impairment; however, the racemate and D-AAB maintained P.I.'s that indicated they had selective anticonvulsant effects. The P.I. for L-AAB of 1.53 indicates much less of a selective anticonvulsant effect than that seen with the racemate and D-AAB. Note that the D-stereoisomer was 1.4

TABLE I

Pharmacological evaluation of the D- and L-stereoisomers of AAB and APB after i.p. administration

	MES-ED_{50}	*TD_{50}*[a]	*P.I.*
Epilepsy Branch			
D,L-AAB	76.54 (66.58–89.04)[b]	453.86 (416.56–501.01)[b]	5.93
D-AAB	54.80 (50.32–59.65)	213.82 (147.71–261.56)	3.90
L-AAB	548.37 (462.57–740.50)	841.38 (691.25–953.59)	1.53
Lilly Research Laboratories			
D,L-AAB	51.0 (44.6–58.6)	>100	–
D-AAB	32.0 (27.49–40.19)	>80	–
D,L-APB	32.1 (27.5–40.2)	>40	–
D-APB	26.4 (21.1–32.0)	>80	–
L-APB	>300	>100 <300	–

[a] TD_{50} from the Epilepsy Branch determined from rotorod; from Lilly Research Laboratories determined from horizontal screen.
[b] 95% Confidence limits.

times more potent than the racemate in the anticonvulsant assay, but 2.1 times more potent in the neurological impairment assay. The fact that it was not twice as potent in the anticonvulsant assay suggests that the presence of the relatively inactive L-stereoisomer in the racemic mixture somehow enhanced the pharmacological activity of the racemate.

D,L-AAB and D-AAB were also compared for anticonvulsant effects at Lilly Research Laboratories. The ED_{50}'s were 51.0 and 32.0 mg/kg, respectively. As was seen with the Epilepsy Branch data, the D-stereoisomer was more potent as an anticonvulsant than the racemate by a factor of 1.6. Since the Epilepsy Branch data had already shown that D,L-AAB and D-AAB had good P.I.'s, no effort was expended to determine an actual TD_{50} on the horizontal screen test at Lilly Research Laboratories.

When the racemate and stereoisomers of APB were studied at Lilly Research Laboratories, a similar picture was seen (lower part of Table I). D,L-APB and D-APB had MES-ED_{50}'s of 32.1 and 26.4 mg/kg, respectively, whereas the L-APB was completely without effect at doses as high as 300 mg/kg. Both D,L-APB and D-APB produced their MES protective effects at doses lower than those which produced neurological impairment, but, because of limitations on compound supply, no TD_{50}'s or P.I.'s were determined. The fact that the D-stereoisomer is not twice as potent as the racemate again suggests that the presence of the inactive L-stereoisomer in the racemate somehow enhances the pharmacological action of the D-stereoisomer.

Fig. 1 shows the comparison of both the D- and L-enantiomers with their racemates at 5 min after i.v. administration. D-APB and D,L-APB produced dose-related anticonvulsant effects with MES-ED_{50}'s of 2.86 (95% confidence interval = 1.93–3.4) and 5.13 (4.29–6.17) mg/kg, respectively. Note that the D-stereoisomer was approximately 1.8 times more potent than the racemate. By contrast, L-APB did not produce protection against MES, but rather a dose-related lethal effect at 10 and 20 mg/kg. Likewise, D-AAB and D,L-AAB produced dose-related anticonvulsant effects, with ED_{50}'s of 31.18 (26.66–38.65) and 70.01 (60.58–88.76), respectively. In contrast, L-AAB was inactive at 80 mg/kg. The ED_{50} of D-APB (2.86 mg/kg) was administered at 5, 10, 20 and 40 min before challenge with MES to determine the duration of activity. The percentages of the 12 animals

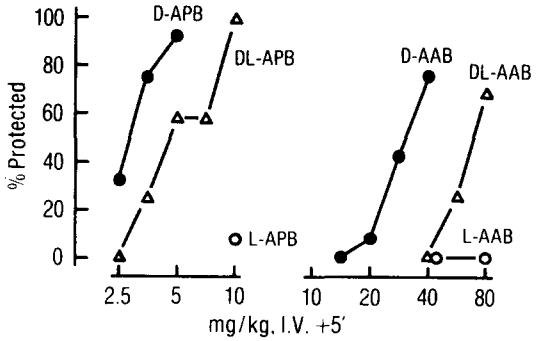

Fig. 1. Effects of i.v. administration of the D-, L-, and D,L-forms of APB and AAB 5 min before MES challenge. Data shown are the % of 12 (except 6 for L-APB) animals protected for each dose.

protected at each of those intervals were 58, 42, 17 and 0, respectively. Thus, after i.v. administration, 5 min was the peak time of activity and the protection became inadequate within 40 min after administration. This suggested that the parent compound, and not a metabolite, was the active compound.

These data suggest that the stereospecificity exhibited after i.p. administration may be a true pharmacodynamic stereospecificity, and would not appear to be due to differences in absorption, distribution or metabolism between the stereoisomers. After i.v. administration, both D-enantiomers were approximately twice as potent as their respective racemates, and both L-enantiomers have no anticonvulsant activity. Thus, this stereospecific anticonvulsant effect differs from that observed with the (S)- and (R)-stereoisomers of LY188544. The 2.2-fold stereospecificity of the (S)-stereoisomer compared to the (R)-stereoisomer of LY188544 was present after oral administration, but there was no stereospecificity after i.v. administration[10].

The marked stereospecificity of these functionalized amino acid derivatives demonstrated by these present data are far superior to stereoselective effects shown for other MES-selective anticonvulsants[1]. Thus, these sets of 'active' and 'inactive' stereoisomers may have great value in studying the biochemical mechanisms of MES-selective anticonvulsants, such as the prototypes phenytoin and carbamazepine.

The clearly less than 2-fold difference in potency in the anticonvulsant assays between the 'active' D-stereoisomers and their respective racemates after i.p. administration suggest that the presence of the L-stereoisomer enhances the pharmacological action of the D-stereoisomer. Such an effect has previously been observed for the D- and L-stereoisomers of propoxyphene after oral, but not subcutaneous administration[12]. That effect was suggested to be due to the inactive isomer saturating the uptake sites for the compound upon first-pass of the liver, which resulted in higher amounts of the active stereoisomer being in the plasma after combined administration of both isomers, than after only administration of the active stereoisomer. Such a mechanism could be acting in this situation since i.p.-administered compounds do undergo a first-pass of the liver before reaching the systemic circulation[11]. The fact that the i.v. comparison with D-APB and D,L-APB was closer to the ideal value of 2 supports this hypothesis. Also, the observation that the ratio of the TD_{50}'s for D-AAB and D,L-AAB was 2.1 is supportive since this hypothesis of liver saturation predicts that, as the amount of drug reaching the liver increases, the percentage of the total taken up by the liver decreases[12].

Other studies have shown that D-APB and L-APB have no protective effects against pentylenetetrazol-induced (85 mg/kg s.c.) clonic seizures[16,17] or *N*-methyl-D-aspartic acid-induced lethality[9]. These results indicate that the benzodiazepine receptor and the *N*-methyl-D-aspartic acid-defined glutamate receptor are probably not involved in the mechanism of action of this class of very interesting anticonvulsants.

The chemical synthesis of these compounds was supported in part by a fellowship awarded to J.D.C. by the American Association of University Women. We thank Dr. D.W. Robertson and Mr. E. Beedle of Lilly Research Laboratories for helpful discussions and Mr. G.D. Gladding, Mr. J.P. Stables, Dr. H.J. Kupferberg and Dr. E.A. Swinyard of the ADD program of the Epilepsy Branch, NINCD, NIH, and Mr. R.R. Lawson of Lilly Research Laboratories for the pharmacological data. Research samples of these compounds can be obtained from Dr. J. David Leander, Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, U.S.A. The Lilly serial numbers are: D,L-APB = LY193326; D-APB = LY248319; L-APB = LY248320; D,L-AAB = LY258499; D-AAB = LY258843; and L-AAB = LY248510.

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