Biochemical Pharmacology, Vol. 23, pp. 2363–2368. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF 2-METHYL 2-ETHYL CAPROIC ACID AND 2-2-DIMETHYL VALERIC ACID ON AUDIOGENIC SEIZURES AND BRAIN GAMMA AMINOBUTYRIC ACID

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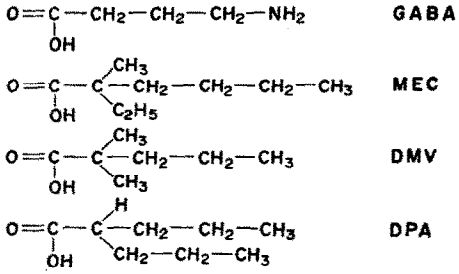
(*Received* 13 *November* 1973; *accepted* 13 *January* 1974)

Abstract—An increase in the level of γ-aminobutyric acid (GABA) in brain was observed after 2-methyl 2-ethyl caproic acid (MEC) and 2-2-dimethyl valeric acid (DMV) treatment, which protects sensitive mice against audiogenic seizures. Enzymatic studies have shown that MEC and DMV inhibit competitively GABA-transaminase with respect to GABA. MEC and DMV are α-substituted fatty acids of the same type as *n*-dipropyl acetic, a structural analogue of GABA.

IN A PREVIOUS study[1] we have shown that *n*-dipropylacetic acid (*n*DPA) protects Swiss albino mice of the Rb line, selected genetically, against audiogenic convulsions. This compound also has at low dose the advantage of facilitating the avoidance conditioning contrary to that in current antiepileptic drugs.[2] During the period of protection against audiogenic fits, the level of γ-aminobutyric acid (GABA) in brain is increased.[1] We have demonstrated *in vitro* that *n*DPA inhibits GABA transaminase purified from mouse brain; this inhibition is competitive with GABA, and non-competitive with α-ketoglutarate.[3]

n-Dipropylacetic acid can be considered as an α-substituted fatty acid of the same type as 2-methyl 2-ethyl caproic acid (MEC) and 2-2-dimethyl valeric acid (DMV). For structural comparison see formulae below. Lespagnol *et al.*[4] have shown the



anti-convulsant properties of MEC and DMV against fits induced by electric shock, pentetrazol and strychnine. We have investigated if the anti-convulsant activity of

Abbreviations used: MEC, 2-methyl 2-ethyl caproic acid; SSA, succinic semi-aldehyde; DMV, 2-2-dimethyl valeric acid; *n*DPA, *n*-dipropylacetic acid; GABA, γ-aminobutyric acid; GABA-transaminase, 4-aminobutyrate-2-ketoglutarate transaminase (EC 2.6.1.19).

these two acids is related to the GABA system by studying their effect on the cerebral GABA content and on GABA-transaminase purified from mouse brain.

MATERIAL AND METHODS

In general, the methods used have been described previously.[3] The following modifications were made.

Extraction and measurement of GABA. GABA was extracted by the method of Okada *et al.*,[5] and the acid soluble fraction was neutralized by a predetermined amount of 20% potassium bicarbonate. The GABA was then measured using a Technicon amino acid analyzer.

GABA-transaminase activity. The activity of GABA-transaminase in the presence of various agents was tested by measurement of the succinic semi-aldehyde (SSA) which was produced by the following reaction:

$$\text{GABA} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{SSA} + \text{glutamate}$$

The SSA was measured by Salvador's modification[6] of the method described by Velluz[7] based on the measurement of the fluorescence of the quinaldine formed after the addition of 3-5-diaminobenzoic acid (Zeiss fluorimeter excitation 405 nm, emission 505 nm). The incubation medium contained 0·1 M Tris–HCl buffer (pH 8), 0·1 mM dithiothreitol and 5×10^{-5} M pyridoxal phosphate.

The concentrations of the substrates were: (a) For the study of K_m (GABA); α-ketoglutarate 5 mM and GABA 10–50 mM. (b) For the study of K_m (α-ketoglutarate); α-ketoglutarate 2 to 15 mM and GABA 50 mM; (c) For the study of the inhibition at the mean cerebral GABA content, GABA 1·3 mM and α-ketoglutarate 1 mM.

The concentrations of the inhibitors for the measurement of K_i were DMV 1·5 mM and MEC 1·2 mM. For the study of the inhibition at the mean value of cerebral GABA content, the GABA transaminase activity was measured in the presence of *n*DPA 1 to 20 mM, DMV $7{\cdot}5 \times 10^{-6}$ to $3{\cdot}3 \times 10^{-3}$ M, MEC $7{\cdot}5 \times 10^{-6}$ to $2{\cdot}6 \times 10^{-3}$ M. The GABA transaminase was purifed about 300-fold from an acetone powder of mouse brain as described previously.[3]

Tests for the protection against audiogenic fits. Our technique has been described previously.[1] MEC and DMV were neutralized with NaOH immediately before they were injected i.p. or subcutaneously in 0·3 ml into a mouse weighing 30 g.

Mathematical analysis. Samples were taken every 30 min up to 2·5 hr from the start of the incubation to determine the transaminase activity. The rate of the reaction was measured by the least squares regression line and the correlation coefficient showed the rate determined by this method is always the initial rate. The functions $1/v = F(1/[s])$ were drawn by the same method from the average values of the reaction rate. The values of the S.E.'s were plotted on the reciprocals of the rates as well as the S.E. of the intersection of these lines with the abscissa for the study of the inhibition in relation to α-ketoglutarate and with the ordinate for study of the inhibition in relation to GABA.

RESULTS

Effects of MEC. An i.p. injection of 100 mg/kg (1·8 m-moles/kg) MEC into Swiss albino mice of the Rb line caused them to become unconscious within 5 min and lose

TABLE 1. PROTECTION AGAINST AUDIOGENIC SEIZURE [100 dB(C) 8000 Hz]

	Time after injection (min)	% Total protection	% Partial protection	% Complete seizure	Number of animals
MEC 200 mg/kg	15	100			16
	30	60		40	16
Intraperitoneal route	60	10	10	80	16
	90	10		90	16
MEC 200 mg/kg	15	90	10		16
Subcutaneous route	30	70	30		16
DMV 280 mg/kg	15	80	20		16
Intraperitoneal route	30	40	40	20	16

Complete seizure: all the signs of the audiogenic seizure; wild running, tonic phase, clonic phase. Partial seizure: wild running only. Total protection: no signs of seizure. All percentage values given to the nearest 10 per cent. The reproducibility of the seizure on sensible mice is 95 per cent.

all reflexes for 30 min. The Jimpy strain with no abnormality of myelinization remained unconscious for 1 hr. This narcotic effect was not seen after a subcutaneous injection; these animals showed normal behaviour.

Table 1 gives the results of the tests of protection against audiogenic fits. It will be seen that MEC protected 90 per cent of the mice 15 min after a subcutaneous injection of 200 mg/kg. We measured the cerebral GABA content at this time and found it increased by 60 per cent which is highly significant (Table 2).

Effects of DMV. An i.p. injection of 280 mg/kg (2·2 m-moles/kg) DMV did not induce the coma seen with MEC. The animals showed normal behaviour. The effects on the audiogenic fits are shown in Table 1. A dose of 280 mg/kg protected 80 per cent of the mice at 15 min. At this time the cerebral GABA had increased by 50 per cent which is highly significant (Table 2).

Inhibition of GABA transaminase in vitro. Figures 1 and 2 illustrate the inhibition caused by MEC and Figs. 3 and 4 give the results for DMV. Both MEC and DMV inhibit GABA-transaminase competitively with respect to GABA with K_i of 3×10^{-4} M and $4{\cdot}5 \times 10^{-4}$ M respectively and non-competitively with respect to α-ketoglutarate with K_i of 6×10^{-4} M and 6×10^{-4} M respectively. At a concentration of GABA close to the mean brain value, a greater than 95 per cent inhibition of the transaminase activity is caused by 2·6 mM MEC and 3·3 mM DMV. At

TABLE 2. GABA LEVEL IN BRAIN AFTER MEC OR DMV TREATMENT

	μmoles/g wet wt	± S.D.	Number of determinations	P
Non-sensitive mice of the same type	1·70	0·20	10	
Genetically sensitive mice	1·66	0·18	4	
Ditto, 15 min after MEC 200 mg/kg subcutaneous route	2·59	0·23	4	<0·0005
Ditto, 15 min after DMV 280 mg/kg i.p. route	2·44	0·14	4	<0·0005

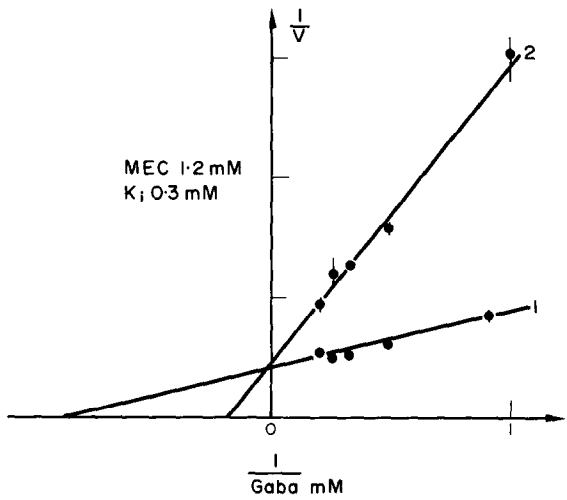


FIG. 1. Lineweaver–Burk plot of GABA-MEC competition. Abscissa: reciprocal of millimolar GABA concn. Ordinate: reciprocal of reaction rate in arbitrary fluorometric units. MEC concn curve 1: 0; curve 2: 1·2 mM. For details of the mathematical analysis see Material and Methods.

the same concentration of GABA, 18 mM *n*DPA inhibited the enzyme activity by only 50 per cent.

DISCUSSION

The administration of 200 mg/kg of MEC i.p. to mice caused them to become unconscious, whilst when it was given subcutaneously their movements remained unchanged. At this dose audiogenic convulsions that were regularly produced in sensitive controls, could be blocked in 90 per cent of the animals 15 min after a subcu-

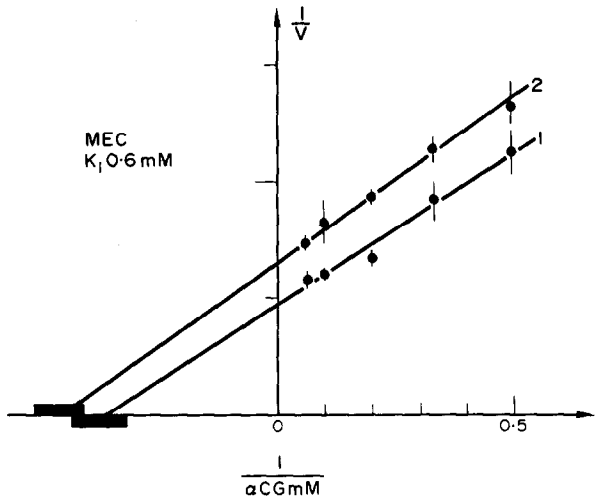


FIG. 2. Lineweaver–Burk plot of α-ketoglutarate-MEC competition. Abscissa: reciprocal of millimolar α-ketoglutarate concn. Ordinate: reciprocal ofreaction rate in arbitrary fluorometric units. MEC concn curve 1: 0; curve 2: 1·2 mM.

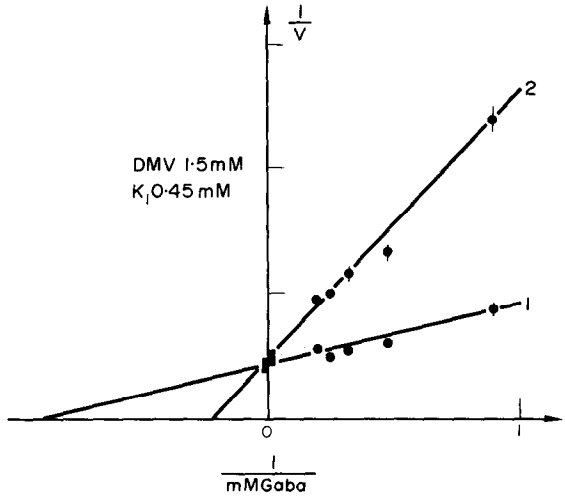


FIG. 3. Lineweaver–Burk plot of GABA-DMV competition. Abscissa: reciprocal of millimolar GABA concn. Ordinate: reciprocal of reaction rate in arbitrary fluorometric units. DMV curve 1: 0; curve 2: 1·5 mM.

taneous injection. Likewise, 15 min after the i.p. injection of DMV, at a dose of 280 mg/kg, 80 per cent of the animals were protected against the convulsions.

In these animals a rise of cerebral GABA was seen similar to that induced by *n*DPA. Our experiments with *n*DPA indicated that its effects are related to its inhibition of GABA-transaminase. We therefore looked at the effects of DMV and MEC on this enzyme. The results show that as for *n*DPA these two acids are competitive inhibitors of GABA-transaminase with respect to GABA but non-competitive with respect to α-ketoglutarate. It is of interest that the K_i GABA of MEC and DMV are

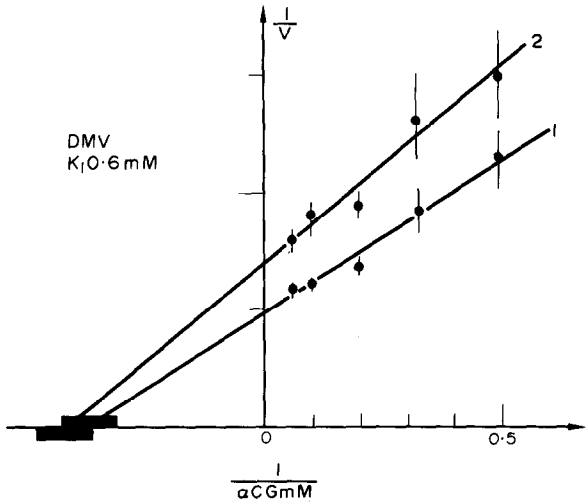


FIG. 4. Lineweaver–Burk plot of α-ketoglutarate-DMV competition. Abscissa: reciprocal of millimolar α-ketoglutarate concn. Ordinate: reciprocal of reaction rate in arbitrary fluorometric units. DMV curve 1: 0; curve 2: 1·5 mM.

3×10^{-4} M and $4{\cdot}5 \times 10^{-4}$ M, respectively, values clearly lower than that of *n*DPA ($1{\cdot}4 \times 10^{-3}$ M).

It has also been demonstrated that 2·6 mM MEC and 3·3 mM DVM produce a 95 per cent inhibition of GABA-transaminase in the presence of a GABA concentration close to that found in the brain, whilst 18 mM *n*DPA only gives a 50 per cent inhibition.

The observation of an anti-convulsant action paralleled by an increase of the GABA content provides more evidence that GABA has an inhibitory function in the central nervous system.[8,9] The mechanism of the anti-convulsant action is most likely due to an inhibition of GABA-transaminase which is accompanied by a rise in the cerebral GABA content. This has opened the way for further research on the anti-convulsants with structures analogous to GABA, and using as a screening test the competitive inhibition of purified GABA-transaminase with respect to GABA.

Acknowledgement—2-Methyl 2-ethyl caproic acid and 2-2-dimethyl valeric acid were kindly provided by Professor Lespagnol (Lille).

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