ELSEVIER

European Journal of Pharmacology 315 (1996) 285–288

ejp

Short communication

Effects of felbamate on veratridine- and K^+-stimulated release of glutamate from mouse cortex

Jayashri Srinivasan, Alan Richens, John A. Davies *

Department of Pharmacology and Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, UK

Received 2 September 1996; accepted 17 September 1996

Abstract

Felbamate is a novel anticonvulsant which may modulate the strychnine-insensitive glycine site of the *N*-methyl-D-aspartate (NMDA) receptor complex. This study examined the effect of felbamate and 5,7-dichlorokynurenic acid on veratridine (20 μM)- and K^+ (60 mM)-stimulated release of amino acids in mouse cortical slices. Felbamate significantly decreased veratridine-induced release of glutamate at 400 μM and 800 μM but had no effect on K^+-stimulated release. 5,7-Dichlorokynurenic acid had no effect on amino-acid release in concentrations up to 200 μM. The inhibitory effect of felbamate on veratridine-induced release of glutamate may be due to inactivation of voltage-sensitive Na^+ channels.

Keywords: Felbamate; 5,7-Dichlorokynurenic acid; Glutamate; Epilepsy; Cortical slice

1. Introduction

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is a novel anticonvulsant (Palmer and McTavish, 1993) and neuroprotectant (Wallis et al., 1992; Wasterlain et al., 1993) licensed for use in the USA in partial seizures with or without secondary generalization and as adjunct therapy in seizures associated with the Lennox-Gastaut syndrome. Trials in the UK and the rest of Europe were halted as a result of an increased incidence of aplastic anaemia and liver dysfunction associated with felbamate therapy (Anonymous, 1994). The mechanism of action of felbamate is not known. However, it inhibits the binding of [^{3}H]5,7-dichlorokynurenic acid, a high-affinity antagonist at the strychnine-insensitive glycine site of the *N*-methyl-D-aspartate (NMDA) receptor complex (McCabe et al., 1993) and the anticonvulsant effects of felbamate are antagonised by glycine (Coffin et al., 1994). The neuroprotective effect of felbamate in rat hippocampal slices is also reversed by glycine (Roi and Panizzon, 1993) and the drug decreases the magnitude of glycine-enhanced NMDA-induced intracellular Ca^{2+} transients in mouse cerebellar cells (White et al., 1995). However, a recent study has shown that at a concentration of 1 mM, felbamate competitively inhibited [^{3}H]dizocilpine binding, indicating it may interact with the channel blocking site of the NMDA receptor; it also induced flickering of NMDA-activated unitary currents, reduced channel open time and burst duration (Subramaniam et al., 1995). In addition, at higher concentrations, felbamate attenuates NMDA-evoked whole cell currents and potentiates γ-aminobutyric acid (GABA)-evoked Cl^- currents in rat hippocampal neurons (Rho et al., 1994). Felbamate, like phenytoin, inhibits sustained-repetitive firing in mouse spinal cord neurons and this suggests it inactivates voltage-sensitive Na^+ channels (White et al., 1992).

In this study, we investigated the effect of felbamate and 5,7-dichlorokynurenic acid on both K^+- and veratridine-induced amino-acid release from slices of mouse cerebral cortex.

2. Materials and methods

2.1. Materials

Felbamate was a gift from Schering-Plough Pharmaceuticals. 5,7-Dichlorokynurenic acid was purchased from Tocris Cookson and veratridine from Sigma. Felbamate and 5,7-dichlorokynurenic were dissolved in dimethyl sulfoxide (DMSO) and diluted in artificial cerebrospinal fluid (aCSF) to give a final concentration of 1% DMSO.

* Corresponding author. Tel.: (44-1222) 742-060; Fax: (44-1222) 747-484.

PII S0014-2999(96)00748-0

Composition of aCSF in mM: NaCl 124, KCl 5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, D-glucose 10 and pH was 7.4. For 60 mM K^+-containing aCSF a corresponding decrease in NaCl was made to maintain osmolarity.

2.2. Cortical slices

Male or female adult BALB/c mice from our colony at the University of Wales College of Medicine, weighing 25–30 g, were used in the study. Animals were killed by cervical dislocation and the brain rapidly removed and placed in ice-cold, gassed (95% oxygen/5% carbon dioxide) aCSF. Coronal cortical slices of 400 μm thickness were cut using a McIlwain tissue chopper and the cortical tissue separated from sub-cortical structures; the hippocampus was not used. Three to four cortical slices weighing 15–20 mg were positioned on a gauze disc and placed in a tissue bath (Barnes et al., 1988) and perfused with gassed aCSF at 2 ml/min at 37°C and were allowed 60 min to equilibrate in aCSF following slicing.

2.3. Neurotransmitter release

Three 2-min samples of perfusate were collected for the measurement of basal amino-acid release. Neurotransmitter release was elicited with two 1-min pulses of veratridine hydrochloride (20 μM) at samples 4 and 14, or two 2-min pulses of K^+ (60 mM) at samples 4 and 14. Felbamate and 5,7-dichlorokynurenic acid in varying concentrations were perfused between samples 7 and 17. All samples were collected on ice and frozen immediately. Slices were weighed and amino-acid release was expressed in pmol/mg tissue/2 min. The amino-acid released in the three consecutive samples collected before the pulses of K^+ or veratridine was averaged to obtain basal release; and the release in the two consecutive samples collected after the pulse of K^+ or veratridine was averaged to obtain stimulated release.

2.4. Amino-acid assay

The amino acids were assayed by high performance liquid chromatography following pre-column derivatization with *o*-phthaldialdehyde and the resulting fluorescence measured (Turnell and Cooper, 1981). The mobile phase consisted of a linear gradient between sodium acetate/tetrahydrofuran and methanol; a 25 cm long reverse-phase C_{18} column was used to separate the amino acids. 200 μl of perfusate was added to 200 μl of homoserine (internal standard) and vortex mixed. Following centrifugation 200 μl were taken and mixed with 50 μl of *o*-phthaldialdehyde. 100 μl of this mixture was then injected onto the column and the resulting fluorescence measured. The following amino acids were assayed: aspartate, glutamate, glycine, taurine, serine and GABA.

2.5. Statistics

Results were calculated as a percentage change from basal release and expressed as mean ± S.E.M. The Student's *t*-test for unpaired data was used to calculate significance levels.

3. Results

3.1. Effect of veratridine (20 μM) and K^+ (60 mM) on endogenous amino-acid release

The basal release of the amino acids in pmol/mg tissue/2 min is given in Table 1. The first pulse of veratridine 20 μM produced an increase in the release of the amino acids (Table 1) with a preferential release of glutamate (8-fold of basal release) and aspartate (4-fold of basal release). The release of the other amino acids was only marginally affected by veratridine. The second pulse of veratridine produced an average release of amino acids of between 60 and 90% of the first pulse.

Table 1
The effect of veratridine (20 μM) and K^+ (60 mM) on endogenous amino-acid release from mouse cortical slices (values are expressed as mean ± S.E.M. in pmol/mg tissue per 2 min)

	Glutamate	Aspartate	GABA	Glycine	Taurine	Serine
Veratridine						
Basal release	3.4 ± 0.9	4.8 ± 1.0	20.9 ± 1.6	17 ± 3.2	16.2 ± 2.8	34.2 ± 7
Pulse 1	28.9 ± 2.7 [c]	19.1 ± 2.9 [b]	28.1 ± 5.5	26.7 ± 8.7	23.1 ± 3.1	41.8 ± 1.9
Pulse 2	18.8 ± 1.8 [c]	10.9 ± 1.5 [b]	23.2 ± 5	21.4 ± 2.4	22.3 ± 2.9	38.1 ± 2.3
Potassium						
Basal release	3.7 ± 0.6	4.7 ± 0.6	28.9 ± 3.8	19.2 ± 2.9	21.9 ± 4.1	36.5 ± 6.6
Pulse 1	39.5 ± 7.3 [c]	20.5 ± 2.9 [c]	39.9 ± 3.2 [a]	32.3 ± 1.5 [a]	28.6 ± 2.3	29.7 ± 1.9
Pulse 2	36.2 ± 7.4 [c]	20.3 ± 3.2 [c]	47.1 ± 6.2 [a]	32.9 ± 2.7 [a]	24.5 ± 1.5	27.7 ± 1.6

$n = 6–9$. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$.

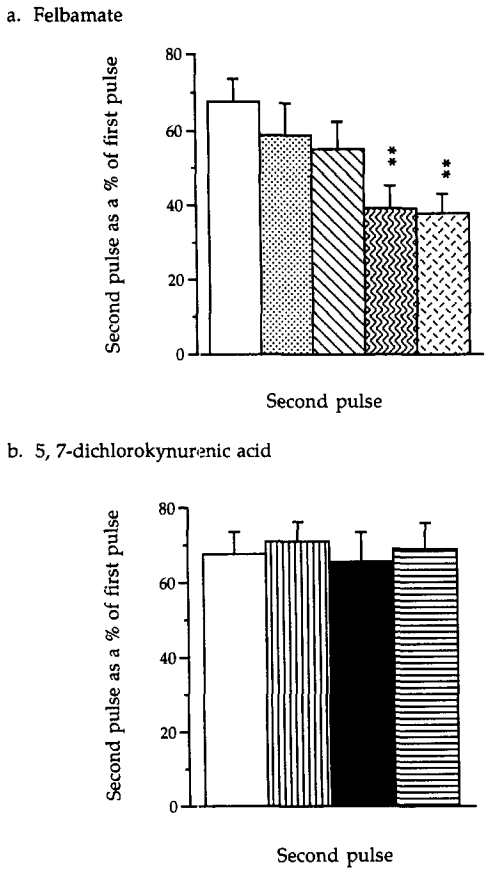

Fig. 1. The effect of (a) felbamate, control (column one), 100 μM (column two), 200 μM (column three), 400 μM (column four) and 800 μM (column five), and (b) 5,7-dichlorokynurenic acid, control (column one), 50 μM (column two), 100 μM (column three) and 200 μM (column four) on veratridine (20 μM)-induced glutamate release. The results are expressed as the mean ± S.E.M. stimulated release in the second pulse as a percentage of the first pulse. $n = 6–9$, ** $P < 0.01$.

The first pulse of K^+ resulted in a significant release of glutamate (8-fold of basal release) and aspartate (4-fold); the release of glycine and GABA were also significantly increased (1.5-fold of basal release) (Table 1). The release of serine and taurine was not significantly altered. The second pulse of K^+ produced an average release of amino acids of between 80 and 90% of the first pulse.

3.2. Effect of felbamate and 5,7-dichlorokynurenic acid on the release of endogenous amino acids

There was no evidence that felbamate in concentrations up to 800 μM or 5,7-dichlorokynurenic acid in concentrations up to 200 μM had any significant effect on the basal release of amino acids.

3.2.1. Veratridine-stimulated release

The second pulse of veratridine produced an average release of glutamate of 67 ± 6% ($n = 9$) of the first pulse. Felbamate reduced this release (Fig. 1a) at concentrations of 400 μM (39.2 ± 6.0%, $P < 0.01$, $n = 6$) and 800 μM (37.9 ± 15.1%, $P < 0.01$, $n = 6$). 5,7-Dichlorokynurenic acid at concentrations between 50 μM and 200 μM had no significant effect on veratridine-induced glutamate release (Fig. 1b). Felbamate and 5,7-dichlorokynurenic acid had no significant effect on the other amino acids released by veratridine including aspartate, serine, taurine, glycine and GABA.

3.2.2. K^+-stimulated release of amino acids

K^+ (60 mM) produced a significant increase in the release of glutamate (approximately 800%) over basal release. The second pulse of K^+ produced an average release of glutamate of 89 ± 7.9% ($n = 7$) when compared to the first pulse. Felbamate at concentrations between 100 μM and 800 μM and 5,7-dichlorokynurenic acid at concentrations between 50 μM and 200 μM had no effect on K^+-stimulated release of glutamate. There was no evidence that felbamate or 5,7-dichlorokynurenic acid had any effect on K^+-induced release of the other amino acids including aspartate, serine, taurine, glycine and GABA.

The solvent DMSO was used at a final concentration of 1% and there was no evidence that DMSO at this concentration had any effect on amino-acid release.

4. Discussion

The results show that felbamate was effective in reducing veratridine-induced glutamate release, while it had no effect on K^+-stimulated release. 5,7-Dichlorokynurenic acid, a potent antagonist at the strychnine-insensitive glycine site of the NMDA receptor, had no effect on veratridine- or K^+-stimulated release of glutamate. Both felbamate and 5,7-dichlorokynurenic acid had no significant effect on the other amino acids assayed.

The mechanism by which an increase in extracellular K^+ stimulates release of excitatory amino acids is secondary to changes in the transmembrane potential resulting in the opening of voltage-sensitive Ca^{2+} channels. The release of neurotransmitters in response to K^+ does not involve Na^+ channels as tetrodotoxin, a potent Na^+ channel blocker, has been shown to be ineffective in inhibiting this release (Dickie and Davies, 1992). Felbamate and 5,7-dichlorokynurenic acid had no effect on K^+-stimulated release.

Veratridine releases neurotransmitters by preventing the inactivation of Na^+ channels; and tetrodotoxin, which blocks Na^+ channels, prevents this release (Minchin, 1980). Drugs such as lamotrigine, which inhibit veratridine-stimulated release of excitatory amino acids but have no effect on K^+-stimulated release, are therefore thought to act by maintaining the inactivation of Na^+ channels (Leach et al., 1986). Felbamate was effective in significantly reducing this release of glutamate in concentrations of 400 μM and 800 μM. This concentration is at the upper end of

the therapeutic range of serum felbamate levels (Rho et al., 1994).

Felbamate inhibits the binding of [^{3}H]5,7-dichlorokynurenic acid, a high affinity antagonist at the strychnine-insensitive glycine site of the NMDA receptor complex (McCabe et al., 1993). Modulation of this glycine site is thought to contribute to the anticonvulsant and neuroprotective properties of felbamate (White et al., 1995). However, 5,7-dichlorokynurenic acid had no effect on K^+- and veratridine-induced release of amino acids. Therefore the effect of felbamate on veratridine-induced release of glutamate is probably unrelated to its affinity at the glycine site of the NMDA receptor.

The inhibition of veratridine-stimulated release of glutamate by felbamate is probably due to inactivation of Na^+ channels and this observation is supported by the evidence that it inhibits sustained-repetitive firing in mouse spinal cord neurons (White et al., 1992). This effect of felbamate on glutamate release probably contributes to its anticonvulsant and neuroprotective efficacy.

Acknowledgements

We are grateful to Schering-Plough Pharmaceuticals for the gift of felbamate.

References

Anonymous, 1994, Felbamate linked to aplastic anaemia; warning issued on drug's use, Am. J. Hosp. Pharm. 51, 2324.

Barnes, S., G.E. Leighton and J.A. Davies, 1988, A novel superperfusion system for the measurement of endogenous glutamate from cerebellar slices, J. Neurosci. Methods. 23 57.

Coffin, V., M. Cohen-Williams and A. Barnett, 1994, Selective antagonism of the anticonvulsant effect of felbamate by glycine, Eur. J. Pharmacol. 256, R9.

Dickie, B.G.M. and J.A. Davies, 1992, Calcium blocking agents and K^+-stimulated release of glutamate from cerebellar slices, Eur. J. Pharmacol. 229, 97.

Leach, M.J., C.M. Marden and A.A. Miller, 1986, Pharmacological studies on lamotrigine, a novel potential antiepileptic drug. II. Neurochemical studies on the mechanism of action, Epilepsia 27, 490.

McCabe, R.T., C.G. Wasterlain, N. Kucharczyk, R.D. Sofia and J.R. Vogel, 1993, Evidence for anticonvulsant and neuroprotective action of felbamate mediated by strychnine-insensitive glycine receptors, J. Pharmacol. Exp. Ther. 264, 1248.

Minchin, M.C.W., 1980, Veratrum alkaloids as transmitter-releasing agents, J. Neurosci. Methods 2, 111.

Palmer, K.J. and D. McTavish, 1993, Felbamate: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in epilepsy, Drugs 45, 1041.

Rho, J., S.D. Donevan and M.A. Rogawski, 1994, Mechanism of action of the anticonvulsant felbamate: opposing effects on *N*-methyl-D-aspartate and gamma-aminobutyric acid receptors, Ann. Neurol. 35, 229.

Roi, A.W. and K. Panizzon, 1993, Glycine reversal of felbamate hypoxic protection, NeuroReport 4, 951.

Subramaniam, S., J. Rho, L. Penix, S. Donevan, R. Fielding and A. Rogawski, 1995, Felbamate block of the *N*-methyl-D-aspartate receptor, J. Pharmacol. Exp. Ther. 273, 878.

Turnell, D.C. and J.D.H. Cooper, 1981, Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography, Clin. Chem. 28, 527.

Wallis, R.A., K.L. Pannizon, M.D. Fairchild and C.G. Wasterlain, 1992, Protective effects of felbamate against hypoxia in the rat hippocampal slice, Stroke 23, 547.

Wasterlain, C.G., L.M. Adams, P.H. Schwartz, H. Hattori, R.D. Soia and J.K. Winchmann, 1993, Posthypoxic treatment with felbamate is neuroprotective in a rat model of hypoxia-ischaemia, Neurology 43, 2303.

White, S.H., H.H. Harold, E.A. Swinyard, G.A. Skeen and R.D. Sofia, 1992, A neuropharmacological evaluation of felbamate as a novel anticonvulsant, Epilepsia 33, 564.

White, S.H., W.L. Harmsworth, R.D. Sofia and H.H. Wolf, 1995, Felbamate modulates the strychnine-insensitive glycine receptor, Epilepsy Res. 20, 41.