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The effect of losigamone (AO-33) on electrical activity and excitatory amino acid release in mouse cortical slices

Jayashri Srinivasan, Alan Richens & [1]John A. Davies

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

1 Losigamone is a novel anticonvulsant the mechanism of action of which is not known. This study investigated the effect of losigamone on spontaneous, NMDA- and AMPA-induced depolarizations in the cortical wedge preparation of the DBA/2 mouse (which are susceptible to sound-induced seizures) and on endogenous amino acid release from BALB/c mouse cortical slices.

2 Cortical wedges exhibit spontaneous depolarizations in magnesium-free medium and losigamone was effective in significantly reducing these spontaneous depolarizations at concentrations of 100 μM and above.

3 NMDA-induced depolarizations were significantly reduced by losigamone at concentrations of 25 μM and above. Losigamone had no effect on AMPA-induced depolarizations.

4 Veratridine (20 μM) and potassium (60 mM) were used to stimulate the release of amino acids from mouse cortex. Veratridine-stimulated release of glutamate was significantly reduced by losigamone at concentrations of 100 μM and above, while potassium-stimulated release was significantly reduced by losigamone at 200 μM.

5 NMDA antagonism and inhibition of excitatory amino acid release may contribute to the anticonvulsant effect of losigamone.

Keywords: Glutamate; NMDA; AMPA; losigamone; epilepsy; cortical slices

Introduction

Losigamone ((±)-5(**R,S**),α(**S,R**)-5-(2-chlorophenyl)hydroxymethyl)-4-methoxy(5H)-furanone) is a novel anticonvulsant the mechanism action of which is not known. It is undergoing Phase II and III clinical trials in patients with partial and secondary generalized seizures. Losigamone belongs to the group of β-methoxy-butenolides which is found in a number of natural substances (Stein, 1995). Losigamone inhibits the tonic hindleg extension produced by electroshock, pentylenetetrazol (PTZ), bicuculline, nicotine and 4-aminopyridine (Stein, 1995). The clonic components of seizures induced by PTZ, bicuculline and picrotoxin are also suppressed by losigamone but it has no effect on the hindleg extension caused by strychnine and picrotoxin (PTX) or the clonic seizures provoked by N-methyl-D-aspartate (NMDA) (Stein, 1995). In the maximal electroshock test (MES) losigamone is more potent than phenytoin and valproate; and in the PTZ test it is more effective than ethosuximide and valproate. Toxicity studies have not produced any significant abnormalities and it does not appear to be teratogenic in animals (Stein, 1995).

In *in vitro* studies losigamone has been shown to reduce the frequency of the spontaneous epileptiform discharges produced by both PTX and perfusion with low-magnesium and low-calcium containing aCSF (Kohr & Heinemann, 1990a; Leschinger *et al*., 1993). It has also been shown to block epileptiform discharges in areas CA1 and CA3 of the hippocampus and in the entorhinal cortex (Kohr & Heinemann, 1990b). Losigamone produces a concentration-dependent increase in chloride uptake in cultured spinal cord neurones and potentiates the effect of exogenous γ-aminobutyric acid (GABA; Dimpfel *et al*., 1995). However, it does not affect [^{3}H]-flunitrazepam or [^{3}H]-GABA binding (Dimpfel *et al*., 1995). Losigamone has been shown, in studies on sustained repetitive-firing in hippocampal-entorhinal cortex slices, to reduce firing (Schmitz *et al*., 1995) as well as reducing excitatory postsynaptic potential (e.p.s.p.) amplitudes, while monosynaptic fast and slow inhibitory postsynaptic potential (i.p.s.ps) were unaffected (Schmitz *et al*., 1995). These results suggest that the drug has a primary mechanism of action on the neuronal membrane. However, unlike phenytoin and carbamazepine which also suppress repetitive firing, losigamone inhibits late recurrent discharges and these are readily inhibited by NMDA antagonists. A study of losigamone on 4-aminopyridine (4-AP)-induced epileptiform activity in the hippocampus showed that losigamone, like other anticonvulsants, partially reversed 4-AP excitation (Yonekawa *et al*., 1995). The same group also studied the effect of anticonvulsants on 4-AP-induced *de novo* synthesis of amino acid neurotransmitters in rat hippocampus and, in common with other drugs that block use-dependent, voltage-sensitive sodium channels such as phenytoin, carbamazepine and lamotrigine, losigamone inhibited 4-AP-induced synthesis of glutamate, aspartate and GABA (Kaptenovic *et al*., 1995).

Genetically epilepsy-prone DBA/2 mice are susceptible to sound-induced seizures (Jobe *et al*., 1991) and cortical wedges from these mice, especially in the absence of Mg^{2+} in the extracellular perfusate, exhibit spontaneous depolarizations (Hu & Davies, 1995). This present study investigated the effect of a racemic mixture of losigamone on spontaneous and NMDA- and α-amino-3-hydroxy-5-methyl-4-isoxazolapropionate (AMPA)-induced depolarizations in cortical wedges prepared from DBA/2 mice. Also the effect of losigamone on potassium- and veratridine-induced release of endogenous amino acids from cortical slices prepared from BALB/c mice was examined.

Methods

Cortical wedge preparation and recording

Cortical wedges were prepared as described previously (Hu & Davies, 1995). In brief, DBA/2 mice aged between 21–30 days, weighing 15–20 g, were killed by cervical dislocation, the brain removed and cortical wedges prepared from coronal slices (500 μm). The wedges were placed in a two-compartment bath with a grease seal isolating the grey cortical matter from the callosum and the callosal side of the preparation was

[1] Author for correspondence.

maintained in a static pool of aCSF. The cortical side was perfused independently with gassed (95% O_2/5% CO_2) aCSF at 2 ml min^{-1} at room temperature (20–22°C) for 60 min to allow the slices to equilibrate. Perfusion of the cortical side was then continued with Mg^{2+}-free aCSF to facilitate NMDA receptor activation. Drugs were applied only to the cortical side of the preparation.

The difference in membrane potential between the two compartments was continuously monitored via Ag/AgCl electrodes and displayed on a BBC Goerz-Metrawatt chart recorder and recorded on a MacLab computer system. The quantification of these epileptiform events was carried out by counting both the number of spontaneous depolarizations and the number of afterpotentials per burst in 5 min epochs. The amplitude of the depolarizations produced by NMDA/AMPA was measured from the peak of the depolarizations to the base line.

Losigamone in 0.2 to 0.5% dimethylsulphoxide (DMSO) was applied at concentrations between 25 and 200 μM for 15 min and the preparation was allowed 30–60 min recovery between drug applications. NMDA or AMPA, in varying concentrations (10 μM to 80 μM), was perfused for 2 min. Losigamone 100 μM was perfused 10 min before and during NMDA or AMPA administration. Responses to NMDA or AMPA were normalized by obtaining at least 3 reproducible responses to the same concentration of the agonist at the beginning of the experiment. Each subsequent response was then calculated as a ratio of the mean of these preliminary responses. These results were then expressed as normalized ratios and compared with control responses.

Cortical slice preparation and amino acid assay

The technique adopted here has been previously described (Srinivasan *et al*., 1995). Briefly, adult BALB/c mice weighing 25–30 g were killed by cervical dislocation. The brain was rapidly removed and placed in ice-cold, gassed aCSF. Coronal cortical slices (400 μm) were cut and the cortical tissue was separated from sub-cortical structures. Three to four cortical slices weighing 15–20 mg were positioned on a gauze disc and placed in a tissue bath and perfused with gassed aCSF at 1 ml min^{-1} at 37°C and allowed 60 min to equilibrate.

Three 2-minute 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) or two 2 min pulses of potassium (60 mM), with an interval of 20 min between the two pulses. The drug to be tested was perfused for 14 min before and during the second pulse. All samples were collected on ice and frozen immediately.

The amino acids were assayed by high performance liquid chromatography following pre-column derivatization with *o*-pthaldialdehyde (Fluka Chemicals and Biochemicals) and the resulting fluorescence measured (excitation filter 254 nm, emission filter 420 nm with a 254 mercury bulb; LDC analytical). The mobile phase consisted of a linear gradient between sodium acetate (0.1 M)/tetrahydrofuran (10 μl l^{-1}) and methanol (Fisons). A 25 cm long reverse-phase C18 column (Jones Chromatography) 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*-pthaldialdehyde; 100 μl of this mixture was then injected on to the column and the resulting fluorescence measured. The amino acids, aspartate, glutamate, glycine, taurine and GABA were assayed.

Drugs

The following drugs were used: losigamone (Willmar Schwabe Arzneimittel); veratridine (Sigma); NMDA and AMPA (Tocris Cookson, U.K.).

The composition of aCSF (in mM) was: 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 potassium-containing aCSF a corresponding decrease in NaCl and for calcium- and magnesium-free aCSF an increase in NaCl was made to maintain osmolarity.

Statistics

Repeated measures were analysed by paired Student's *t* test. Multiple comparisons were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *post-hoc* test.

Results

Losigamone on electrical activity

Effect of losigamone on spontaneous depolarizations and afterpotentials Cortical slices from DBA/2 mice, exhibit spontaneous depolarizations when perfused with magnesium-free aCSF and the majority of these depolarizations show afterpotentials. The frequencies of these depolarizations in magnesium-free medium were between 3–10 min^{-1} and were relatively fast in character with rise times of 90–120 ms and a duration of 1–5 s. The depolarizations increased in frequency for the first 2 h of perfusion with magnesium-free medium but were stable thereafter for about 6 h. Afterpotentials, when present, varied in frequency between 1–6 on each depolarization.

The frequency of spontaneous depolarizations expressed as a percentage of baseline responses was $102.2 \pm 1.1\%$ ($n = 10$) before drug treatment. Losigamone in concentrations of 100 μM ($P < 0.05$) and 200 μM ($P < 0.01$) significantly reduced the frequency of spontaneous depolarizations (Table 1). At 400 μM, losigamone almost completely inhibited the depolarizations ($P < 0.001$); and the few depolarizations seen were of reduced amplitude (Figure 1). The reduction in frequency was seen within 10 min of application of losigamone and the responses returned to control levels within 20–30 min after reinstatement of drug-free aCSF.

Losigamone significantly reduced afterpotentials at 25 μM ($P < 0.001$), and at concentrations of 50 μM and above the afterpotentials were completely inhibited.

Effect of losigamone on NMDA- and AMPA-induced depolarizations NMDA at varying concentrations (10 μM to 80 μM) was used to produce depolarizations. The effect of varying concentrations of losigamone on NMDA (10 μM) was calculated as normalized ratios of control values. Losigamone significantly inhibited depolarizations produced by NMDA at concentrations of 50 μM ($P < 0.01$) and 100 μM ($P < 0.01$, Figure 2). Losigamone in concentrations up to 200 μM had no effect on AMPA-induced depolarizations (Figure 3).

Losigamone on amino acid release

Effect of veratridine 20 μM and potassium 60 mM on amino acid release The basal release of glutamate and aspartate was

Table 1 Effect of losigamone on spontaneous depolarizations and afterpotentials/burst in the cortical wedge preparation expressed as a percentage of baseline responses

	Spontaneous depolarizations			*After potentials/burst*		
	n	*Mean* (%)	P	n	*Mean* (%)	P
Control	10	102.1 ± 1.1		6	104.9 ± 1.9	
Losigamone						
25 μM	10	96.9 ± 6.5	NS	6	37 ± 6.5	<0.01
50 μM	10	92.5 ± 8.1	NS	6	0	
100 μM	10	67.7 ± 6.5	<0.05	6	0	
200 μM	10	49.2 ± 4.2	<0.01	6	0	
400 μM	4	4.4 ± 0.9	<0.001	4	0	

Results are presented as mean ± s.e.mean.

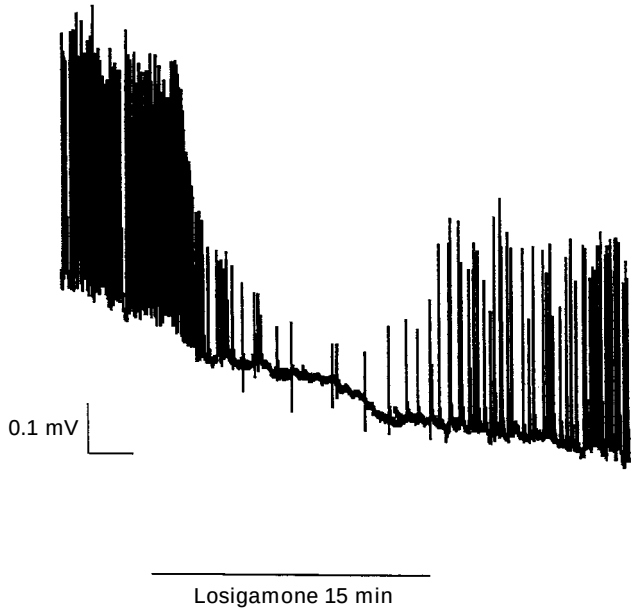

Figure 1 A representative trace of the effect of losigamone 400 μM on spontaneous depolarizations of the cortical wedge preparation from a DBA/2 mouse.

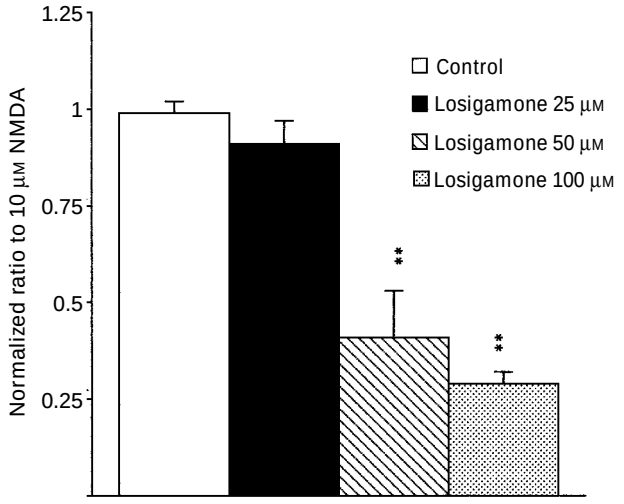

Figure 2 The effect of losigamone on depolarizations induced by NMDA 10 μM. The results are expressed as the normalized ratios to control responses (NMDA 10 μM); $n=6-8$. $^{**}P<0.01$ (one-way ANOVA followed by Dunnett's test).

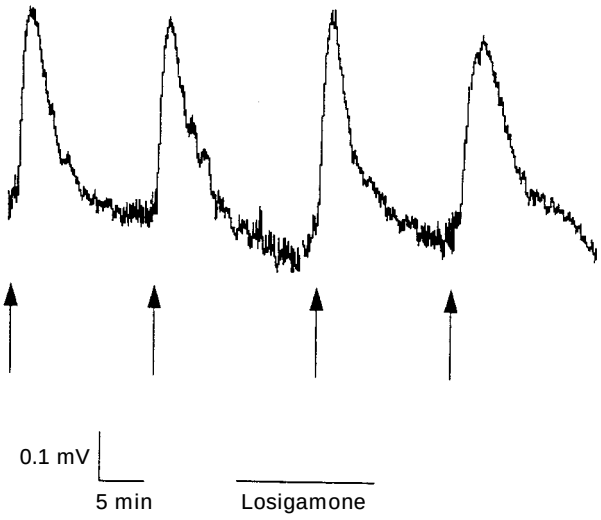

Figure 3 Effect of losigamone 100 μM on AMPA (10 μM)-induced depolarizations (arrows) in the cortical wedge preparation from a DBA/2 mouse.

3.7 ± 0.6 and 4.7 ± 0.6 pmol mg^{-1} tissue in 2 min, respectively. Veratridine (20 μM) and potassium (60 mM) both produced significant increases in the release of glutamate (700% and 800%, respectively) and aspartate (300% and 400%, respectively).

Effect of losigamone on amino acid release There was no evidence that losigamone in concentrations up to 200 μM had any effect on the basal release of amino acids.

The second pulse of veratridine resulted in an average glutamate release of $77.6\pm2.4\%$ ($n=6$) of the first pulse, while the second pulse of potassium resulted in an average glutamate release of $98.9\pm7.9\%$ ($n=6$) of the first pulse. Veratridine-stimulated release of glutamate was reduced significantly by losigamone at concentrations of 100 μM and 200 μM ($P<0.01$, Figure 4a) whilst potassium-stimulated release of glutamate was reduced significantly by losigamone at 200 μM ($P<0.01$, Figure 4b).

The second pulse of veratridine resulted in an average aspartate release of $79.1\pm4.7\%$ ($n=6$) of the first pulse, while the second pulse of potassium resulted in an average release of $93.3\pm7.5\%$ ($n=6$) of the first pulse. Veratridine- and potassium-stimulated release of aspartate was inhibited significantly by losigamone 200 μM ($P<0.01$, Figure 5).

Losigamone had no effect on the release of GABA, glycine, serine or taurine (data not shown).

Discussion

The results presented here show that losigamone significantly reduced the frequency of spontaneous depolarizations at concentrations of 100 μM and above, and the frequency of associated afterpotentials at 25 μM and above, in the cortical wedge preparation from DBA/2 mice. It also had an inhibitory effect on NMDA-induced depolarizations but had no effect on AMPA-induced depolarizations.

DBA/2 mice are genetically epilepsy-prone and this behaviour is age related, in that mice of 20–30 days of age are far more susceptible to seizures than younger or older mice. In our colony, 96% of mice at this age respond to a 110 dB sound with characteristic wild-running and tonic clonic seizures (Abila *et al*., 1993). As we have previously shown (Hu & Davies, 1995), 90% of slices prepared from animals aged between 20–30 days exhibit spontaneous depolarizations in magnesium-free medium. The role of NMDA receptors in seizure initiation and propagation is well recognized (Meldrum, 1992) and potential anticonvulsant compounds, acting on diverse sites of the NMDA receptor, have been shown to reduce burst frequency and the number of afterpotentials in the rat cortical wedge preparation (Aram *et al*., 1989). This is suggestive that NMDA receptor complex activation is involved in these epileptiform events in this *in vitro* preparation. Anticonvulsant compounds acting at other sites, such as σ ligands and GABA agonists have also been shown to be inhibitory in the cortical wedge preparation (Horne *et al*., 1986; Palmer *et al*., 1992).

The inhibitory effect of losigamone on spontaneous depolarizations, associated afterpotentials and NMDA-induced depolarizations shown in this study is suggestive of NMDA-receptor antagonism. It is not possible to ascertain from the above results the site of action of losigamone on the NMDA receptor, as drugs acting at different sites on the NMDA receptor would be capable of reducing spontaneous and

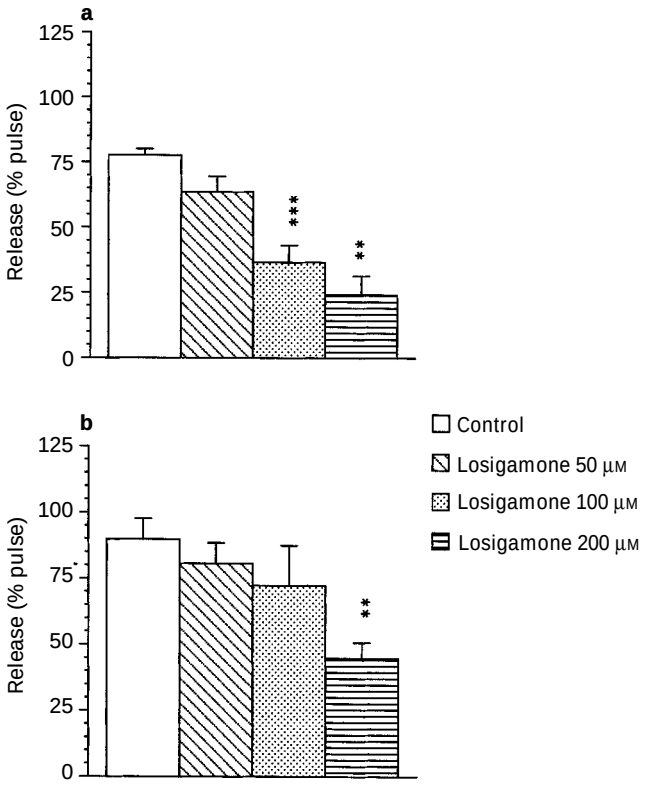

Figure 4 The effect of losigamone on (a) veratridine- (20 μM) and (b) potassium-induced (60 mM) release of glutamate. The results are expressed as the mean ($\pm$s.e.mean) stimulated release in the second pulse as a percentage of the first pulse; $n=4-6$. $^{**}P<0.01$ and $^{***}P<0.001$ (one-way ANOVA followed by Dunnett's test).

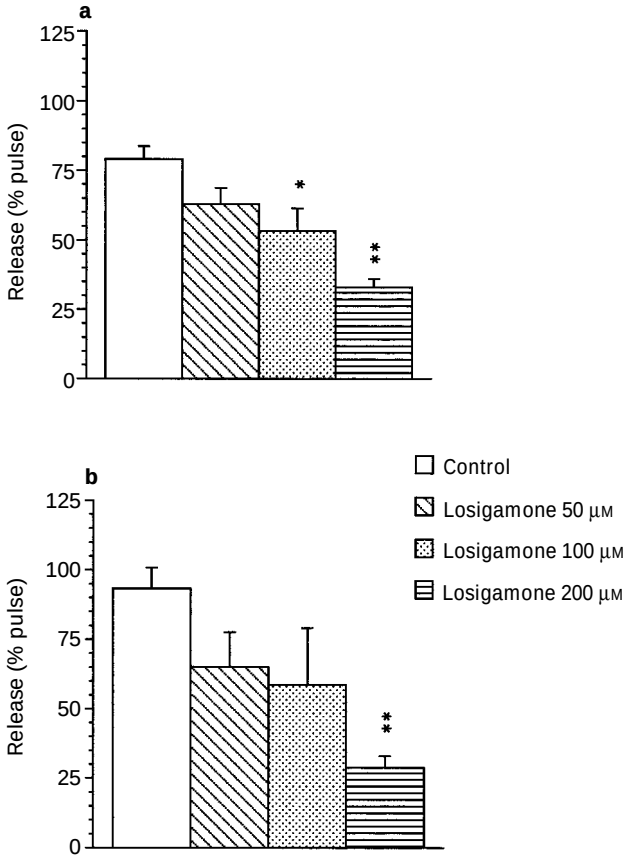

Figure 5 The effect of losigamone on (a) veratridine- (20 μM) and (b) potassium-induced (60 mM) release of aspartate. The results are expressed as the mean ($\pm$s.e.mean) stimulated release in the second pulse as a percentage of the first pulse; $n=4-6$. $^{*}P<0.05$ and $^{**}P<0.01$ (one-way ANOVA followed by Dunnett's test).

NMDA-induced depolarizations. These present results do not support those of Stein (1995), who showed no effect of losigamone on NMDA-induced convulsions in mice.

In addition, this study showed that losigamone reduced veratridine-induced release of glutamate at concentrations of 100 μM and above, and potassium-induced release of glutamate and potassium- and veratridine-induced release of aspartate at concentrations of 200 μM. As previously shown, potassium-stimulated release is calcium-dependent while veratridine-stimulated release is only partially affected by removal of calcium from the medium (Srinivasan *et al*., 1995).

The mechanism by which an increase in extracellular potassium stimulates release of excitatory amino acids is secondary to changes in the transmembrane potential, resulting in the opening of voltage-sensitive calcium channels. The release of neurotransmitters in response to potassium does not involve sodium channels as tetrodotoxin, a potent sodium channel blocker, has been shown to be ineffective in inhibiting this release (Dickie & Davies, 1992). Activation of the NMDA receptor by the endogenous ligand initiates a positive feedback loop occurring at the synaptic level, which acts on the presynaptic terminal to increase the release of transmitter. There are various modulators which have been shown to be involved in this positive feedback response, including nitric oxide (Rowley *et al*., 1993; Montague *et al*., 1994) and arachidonic acid (Dickie *et al*., 1994). Drugs which are NMDA receptor antagonists may prevent this positive feedback resulting in reduced neurotransmitter release and, conversely, NMDA has been shown to stimulate glutamate release from mouse cortical slices (Rowley *et al*., 1993). The glutamate released from the cortical slice preparation probably comes from the glutamatergic neurones, which form association and commissural fibres, and the dendrites of these neurones possess NMDA receptors (Huntley *et al*., 1994). If activation of these receptors is blocked by an NMDA antagonist, as is probably the case with losigamone, then augmentation of glutamate release by NMDA receptor stimulation will not occur. The differences in concentration of losigamone between that required to reduce potassium-stimulated release of glutamate and that which reduced NMDA-induced depolarizations is probably due to the nature of the stimulation. We have previously found (Hu & Davies, 1995) that exogenous application of NMDA was reduced by remacemide at lower concentrations than was potassium-stimulated glutamate release (Srinivasan *et al*., 1995), which, by nature of the methodology, is through endogenous mechanisms.

Veratridine releases neurotransmitters by preventing the inactivation of sodium channels and tetrodotoxin, which blocks sodium channels, prevents this release (Levi *et al*., 1980; Minchin, 1980). Drugs such as lamotrigine, which inhibit veratridine-stimulated release of excitatory amino acids but have no effect on potassium-stimulated release, are therefore thought to act by maintaining the inactivation of sodium channels (Leach *et al*., 1986). Recently published results are compatible with the observation that losigamone is a sodium channel blocker (Schmitz *et al*., 1995). Losigamone has been shown in studies on sustained repetitive-firing in hippocampal-entorhinal cortical slices (a test which involves the activation of voltage-operated sodium channels) to reduce firing (Schmitz *et al*., 1995). In addition, losigamone reduced e.p.s.p. amplitudes while monosynaptic fast and slow i.p.s.ps were unaffected. These results suggest that the drug has a primary mechanism of action on the neuronal membrane. However, unlike phenytoin and carbamazepine which also suppress repetitive firing, losigamone inhibits late recurrent discharges and these are readily inhibited by NMDA antagonists. Losigamone, in common with other drugs that block use-dependent voltage-

sensitive sodium channels, such as phenytoin, carbamazepine and lamotrigine, inhibited 4-AP-induced synthesis of glutamate, aspartate and GABA (Kaptenovic *et al*., 1995). The inhibitory effect of losigamone on veratridine-induced release of glutamate and aspartate could therefore be secondary to sodium channel blockade. However, the effect of losigamone in reducing potassium-stimulated release of glutamate and aspartate, is not a sodium channel effect as TTX does not reduce release. The action of losigamone probably involves NMDA receptor antagonism as we have previously shown that dizocilpine (MK-801) reduced potassium-stimulated release of glutamate (Srinivasan *et al*., 1995).

The NMDA receptor antagonism and inhibition of excitatory amino acid release demonstrated here could be relevant to the anticonvulsant effect of losigamone.

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References

ABILA, B., RICHENS, A. & DAVIES, J.A. (1993). Anticonvulsant effects of extracts of the West African black pepper, *Piper guineense*. *J. Ethnopharmacol*., **39,** 113 – 117.

ARAM, J.A., MARTIN, D., TOMCZYK, M., MILLAR, J., POHLER, G. & LODGE, D. (1989). Neocortical epileptogenesis in vitro: studies with NMDA, PCP, sigma and dextromethorphan receptor ligands. *J. Pharmacol. Exp. Ther*., **248,** 320 – 328.

DICKIE, B.G.M., ANNELS, S.J. & DAVIES, J.A. (1994). Effects of cyclooxygenase inhibition upon glutamate release from rat cerebellum. *NeuroReport*, **5,** 393 – 396.

DICKIE, B.G.M. & DAVIES, J.A. (1992). Calcium blocking agents and potassium-stimulated release of glutamate from cerebellar slices. *Eur. J. Pharmacol*., **229,** 97 – 99.

DIMPFEL, W., CHATTERJEE, S.S., NOLDNER, M. & TICKU, M.K. (1995). Effects of the anticonvulsant losigamone and its isomers on the $GABA_A$ receptor system. *Epilepsia*, **36,** 983 – 989.

HORNE, A.L., HARRISON, N.L., TURNER, J.P. & SIMMONDS, M.A. (1986). Spontaneous paroxysmal activity induced by zero magnesium and bicuculline: suppression by NMDA antagonists and GABA mimetics. *Eur. J. Pharmacol*., **122,** 231 – 238.

HU, R.Q. & DAVIES, J.A. (1995). The effect of the desglycinyl metabolite of remacemide on cortical wedges prepared from DBA/2 mice. *Eur. J. Pharmacol*., **287,** 251 – 256.

HUNTLEY, G.W., VICKERS, J.C. & MORRISON, J.H. (1994). Cellular and synaptic localization of NMDA and non-NMDA receptor subunits in neocortex: organizational features related to cortical circuitry, function and disease. *Trends Neurosci*., **17,** 536 – 543.

JOBE, P., MISHRA, P., LUIG, N. & DAILEY, W. (1991). Sope and contribution of genetic models to an understanding of epilepsy. *Neurobiology*, **6,** 183 – 220.

KAPTENOVIC, I.M., YONEKAWA, W. & KUPFERBERG, H.J. (1995). The effects of anticonvulsant compounds on 4-aminopyridine-induced *de novo* synthesis of neurotransmitter amino acids in rat hippocampus in vitro. *Epilepsy Res*., **20,** 113 – 120.

KOHR, G. & HEINEMANN, U. (1990a). Effects of tetronic acid derivatives AO-33 (losigamone) and AO-78 on epileptiform activity in rat hippocampal slices. *Epilepsy Res*., **7,** 49 – 55.

KOHR, G. & HEINEMANN, U. (1990b). Anticonvulsant properties of tetronic acid derivatives on picrotoxin-induced epileptiform activity in rat hippocampal slices. *Neurosci. Lett*., **112,** 43 – 47.

LEACH, M.J., MARDEN, C.M. & MILLER, A.A. (1986). Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia*, **27,** 490 – 497.

LESCHINGER, A., STABEL, J., IGELMUND, P. & HEINEMAN, U. (1993). Pharmacological and electrographic properties of epileptiform activity induced by elevated K^+ and lowered Ca^{2+} and Mg^{2+} concentration. *Exp. Brain Res*., **96,** 230 – 240.

LEVI, G., GALLO, V. & RAITERI, M. (1980). A re-evaluation of veratridine as a tool for studying the depolarisation-induced release of neurotransmitter from nerve-endings. *Neurochem. Res*., **5,** 281 – 295.

MELDRUM, B.S. (1992). Excitatory amino acids in epilepsy and potential novel therapies. *Epilepsy Res*., **12,** 189 – 196.

MINCHIN, M.C.W. (1980). Veratrum alkaloids as transmitter-releasing agents. *J. Neurosci. Meth*., **2,** 111 – 112.

MONTAGUE, P.R., GANCAYCO, C.D., WINN, M.J., MARCHASE, R.B. & FRIEDLANDER, M.J. (1994). Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. *Science*, **263,** 973 – 977.

PALMER, A.J., ZEMAN, S. & LODGE, D. (1992). Methohexitone antagonises kainate and epileptiform activity in rat neocortical slices. *Eur. J. Pharmacol*., **221,** 205 – 209.

ROWLEY, H.L., ELLIS, Y. & DAVIES, J.A. (1993). Age-related effects of NMDA-stimulated concomitant release of nitric oxide and glutamate in cortical slices prepared from DBA/2 mice. *Brain Res*., **613,** 49 – 53.

SCHMITZ, D., GLOVELLI, T. & HEINEMANN, U. (1995). Effect of losigamone on synaptic potentials and spike frequency habituation in rat entorhinal cortex and hippocampal CA1 neurones. *Neurosci. Lett*., **200,** 141 – 143.

SRINIVASAN, J., RICHENS, A. & DAVIES, J.A. (1995). The effect of the desglycinyl metabolite of remacemide hydrochloride (FPL 12495AA) and dizocilpine (MK-801) on endogenous amino acid release from mouse cortex. *Br. J. Pharmacol*., **116,** 3087 – 3092.

STEIN, U. (1995). Losigamone. In *Antiepileptic Drugs*. ed. Levy, R., Mattson, R. & Meldrum, B. pp. 1025 – 1034. New York: Raven Press.

YONEKAWA, W.D., KAPETANOVIC, I.M. & KUPFERBERG, H.J. (1995). The activity in rat hippocampus in vitro. *Epilepsy Res*., **20,** 137 – 150.

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