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The anticonvulsant effects of the enantiomers of losigamone

[1]Francis A. Jones & *,[1]John A. Davies

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

1 Losigamone is a novel anticonvulsant undergoing phase III clinical trials in patients with partial and secondary generalized seizures. This study investigated the effects of the **S**(+)- and **R**(−)-enantiomers of losigamone on endogenous amino acid release from BALB/c mouse cortical slices, spontaneous depolarizations in the cortical wedge preparation of the DBA/2 mouse and audiogenic seizures in DBA/2 mice.

2 **S**(+)-losigamone (100 and 200 μM) significantly reduced both potassium- and veratridine-elicited release of glutamate and aspartate from cortical slices. **R**(−)-losigamone had no effect on release at concentrations up to 400 μM.

3 Cortical wedges exhibit spontaneous depolarizations when perfused with magnesium-free artificial cerebrospinal fluid. **S**(+)-losigamone significantly reduced these depolarizations at 50–200 μM whilst **R**(−)-losigamone had a significant effect at 200–800 μM.

4 DBA/2 mice are susceptible to audiogenic seizures and **S**(+)-losigamone dose-dependently (5, 10 and 20 mg kg^{-1}, i.p.) significantly inhibited clonic/tonic convulsions with 91% of the mice protected at 20 mg kg^{-1}. There was no protection at 20 mg kg^{-1} with **R**(−)-losigamone.

5 These results, from both *in vitro* and *in vivo* experiments, confirm that the pharmacological activity profiles of the two losigamone enantiomers are not identical and suggest further that excitatory amino acid-mediated processes are involved in the mode of action of **S**(+)-losigamone whereas **R**(−)-losigamone does not possess such properties. For the treatment of neurological conditions involving exaggerated excitatory amino acid function the use of **S**(+)-losigamone might therefore be more effective clinically than losigamone or its **R**(−)-enantiomer.

Keywords: Anticonvulsants; losigamone enantiomers; brain slices; amino acid release; audiogenic seizures

Abbreviations: aCSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DMSO, dimethyl sulphoxide; GABA, γ-aminobutyric acid; HPLC, high performance liquid chromatography; NMDA, N-methyl-D-aspartate; TTX, tetrodotoxin

Introduction

Epilepsy remains one of the most significant clinical challenges in neurology, it comes in many forms, can be very disabling, and can effect people of all ages (Hauser & Annegers, 1993). The balance between the activity of inhibitory γ-aminobutyric acid (GABA) and excitatory L-glutamate and L-aspartate neurotransmitter amino acids is important for normal neurological function and may play a crucial role in the pathogenesis and potential treatment of epilepsy (Loscher, 1993). Research over the last 50 years has resulted in a number of antiepileptic drugs becoming available (Sander, 1998), but only 80% of patients become seizure-free on the currently available drugs (Richens & Perucca, 1993). It is therefore clear that there remains a need for new and better antiepileptic drugs.

Losigamone, ((±)-5(**R**,**S**),α(**S**,**R**)-5-((2-chlorophenyl)hydroxyl-methyl)-4-methoxy(5H)-furanone), has shown potential for fulfilling this need, it has been demonstrated to possess an unique anticonvulsant activity profile and has excellent tolerability (Stein *et al*., 1991). It was chosen following synthesis and pharmacological screening of analogues and derivatives of naturally occurring five and six membered lactones occurring in various *Piper* species (Stein, 1995). This work led to the identification of several tetronic acid derivatives which exhibited anticonvulsant activity in several animal models such as maximal electroshock, pentylenetetrazol, bicuculline and 4-aminopyridine induced convulsions (Stein *et al*., 1991).

The observed pharmacological activity profile of losigamone indicates that modulation of GABA and excitatory amino acid-mediated processes could be involved in its mode of action (Chatterjee & Noldner, 1997). Losigamone has been shown to potentiate GABA-mediated responses and reduce epileptiform activity induced by chloride channel antagonists at low micromolar concentrations (Dimpfel *et al*., 1995). Both the enantiomers were shown to possess GABA-potentiating properties with the R(+)-enantiomer being the more potent (Dimpfel *et al*., 1995). Although losigamone does not inhibit NMDA-induced convulsions in mice (Stein *et al*., 1991), it has been shown to block N-methyl-D-aspartate (NMDA)-induced depolarizations in mouse cortical wedges and also to reduce both veratridine- and potassium-induced release of excitatory amino acids from cortical slices (Srinivasan *et al*., 1997). In addition, losigamone has also been reported to inhibit the *de novo* synthesis of 4-aminopyridine-induced neurotransmitter amino acids in rat hippocampus (Kapetanovic *et al*., 1995).

Losigamone is a racemic mixture of two threo-enantiomers **S**(+)-losigamone and **R**(−)-losigamone. In this present study the two enantiomers were tested on amino acid release and on spontaneous depolarizations from cortical slices. In addition, both the enantiomers were tested for anticonvulsant activity in genetically epilepsy prone DBA/2 mice.

*Author for correspondence.

Methods

Cortical slice preparation and amino acid assay

The technique used for the preparation of cortical slices was previously described by Srinivasan *et al.* (1995). Briefly, BALB/c mice aged between 21 and 50 days were killed by cervical dislocation followed by decapitation, the whole brain was rapidly removed before being immersed in gassed (95% oxygen/5% carbon dioxide) ice-cold artificial cerebrospinal fluid (aCSF).

Coronal slices (400 μm) were cut and separated using fine brushes, cortical tissue was then separated from the subcortical structures. Approximately three of these slices were transferred to a previously weighed gauze disc and placed in a tissue bath and perfused with gassed aCSF at 1 ml min^{-1} (37°C). Between 45–60 min was allowed for equilibration as amino acid release following slicing has been shown to plateau after approximately 40 min (Barnes *et al.*, 1988).

Experiments were carried out starting approximately 60 min after slicing and 2 min samples of perfusate were collected continuously for a further 48 min. Three 2 min samples of perfusate were collected to ascertain the basal (unstimulated) levels of amino acid release. Amino acid release was stimulated with either two 2 min pulses of potassium chloride (60 mM) or two 1 min pulses of veratridine hydrochloride (20 μM) with 20 min between the two pulses. Drugs were made up in aCSF after dissolving in dimethyl sulphoxide (final concentration of DMSO was $<0.05\%$). Drug perfusion occurred 14 min prior to and during the second pulse of stimulation. The samples were collected on ice and frozen immediately for subsequent analysis.

Amino acid samples were assayed by high performance liquid chromatography (HPLC) following pre-column derivatization with *o*-pthaldialdehyde (Fluka Chemicals and Biochemicals) with subsequent fluorometric detection (excitation filter 254 nm, emission filter 420 nm with a 254 nm mercury bulb; LDC analytical) for the following amino acids; aspartate, glutamate, glutamine, serine, glycine, taurine and GABA (Turnell & Cooper, 1981). The mobile phase consisted of a linear gradient between sodium acetate (0.1 M), tetrahydrofuran (10 ml l^{-1}) and methanol (Fisons). A 25 cm reverse-phase C_{18} octadecyl 5 μm column (Jones Chromatography) was used to separate the amino acids. Two hundred μl of the sample was mixed with 200 μl of homoserine (internal standard) then vortex mixed. Following centrifugation 200 μl was taken and added to 50 μl of *o*-pthaldialdehyde, and finally 150 μl of this mixture was then injected onto the column and the resulting fluorescence measured and displayed on a chart recorder (BBC Goertz-Metrawatt).

Cortical wedge preparation

Cortical wedges were prepared as previously described by Hu & Davies (1995). Briefly, brains from male or female DBA/2 mice aged between 21 and 42 days were used. Coronal slices (500 μm) were cut and from these slices wedges of cerebral cortex, corpus callosum and striatum were prepared. Cuts of approximately 4 mm length were made close to the midline 2 mm apart at the cortex and tapering to 1 mm wide at the striatum.

One wedge was immediately transferred to a two compartment tissue bath, where the grey cortical matter was separated from the callosum and striatum by a thin wall of silicon grease. The compartment containing the callosal side of the wedge was filled with aCSF and the second compartment, the cortical side, was continuously perfused with gassed aCSF at 2 ml min^{-1}. One hour was allowed for the tissue to equilibrate at room temperature (20–22°C). Drugs were perfused across the cortical side of the wedge. The direct current potential between the two compartments was continuously monitored using silver/silver chloride electrodes, amplified (Flyde 2601A) and displayed on a chart recorder (BBC Goertz-Metrawatt) and also recorded on a MacLab computer system at (AD Instruments, Hastings, U.K.).

Audiogenic seizures with DBA/2 mice

Male DBA/2 mice aged 21 days were obtained from Harlan Olac (U.K.) and housed for 3 days prior to testing under controlled conditions (temperature 21–22°C; lighting on 0600–1800 h). Food and water were available *ad libitum*. The testing chamber consisted of a clear perspex box 58 cm square and 30 cm high with an electric door bell attached to the inner upper surface. The bell produced a sound of approximately 10 KHz (110–120 dB), and the resulting audiogenic seizure consisted of wild running and clonic seizure followed by tonic hindlimb extension. Mice were tested individually and kept in a room distant from the experimental room. Each animal was exposed to this stimulus for 60 s, with tonic hindlimb extension being considered the end-point of the seizure. Each animal was used once only. Animals that did not show tonic hindlimb extension were considered protected.

Drugs were dissolved in DMSO, made up in 0.9% sterile saline and administered intraperitoneally 1 h prior to testing. Control mice were treated with saline containing 0.5% DMSO.

Drugs and chemicals

The following drugs were used: the two enantiomers of losigamone, **S**(+)-losigamone (AO-242) and **R**(−)-losigamone, (AO-294) and the racemic mixture, (AO-33, Willmar Schwabe, Arzneimittel, Karlsruhe): veratridine (Sigma).

The 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 the pH was 7.4. The composition of the 60 mM potassium aCSF was adjusted to maintain osmolarity with a corresponding decrease in NaCl, similarly there was an increase in NaCl in magnesium-free aCSF.

Statistics

Amino acid release data were calculated as pulse 1/pulse 2 percentages and expressed as mean ± s.e.mean Student's *t*-test (unpaired) was used to calculate significance levels. One-way ANOVA followed by the Student-Newman-Keuls test was used to calculate drug effects.

Magnesium-free aCSF-induced depolarizations in the wedge preparation were counted over three 5 min periods: 5 min immediately prior to drug perfusion (first period), for the final 5 min of 15 min drug perfusion (second period) and then for 5 min of 15 min after the end of drug perfusion (third period). Values from the second and third periods were then compared to the value from the first period using Student's *t*-test (paired) to calculate significance levels.

Data from the audiogenic seizure study were analysed using Chi-square test with Fishers exact test to compare drug treatments with control data.

Results

Effects of veratridine 20 μM and potassium 60 mM on amino acid release

The basal release of glutamate and aspartate was 4.14 ± 0.85 and 3.85 ± 0.53 pmoles mg tissue^{-1} 2 min^{-1} respectively. Veratridine (20 μM) and potassium (60 mM) both produced significant increases in release of glutamate (500 and 600% respectively) and aspartate (300 and 400% respectively; $n = 8-10$, $P < 0.001$ in all cases).

*Effects of **S**(+)-losigamone on amino acid release*

The second pulse of potassium resulted in an average aspartate release of $94.4 \pm 2.8\%$ ($n = 6$) of the first pulse, and an average second pulse release of glutamate of $88.4 \pm 1.1\%$ ($n = 6$). Potassium-stimulated release of both aspartate and glutamate were significantly reduced by **S**(+)-losigamone (Figure 1a) at concentrations of 100 μM ($P < 0.05$ and $P < 0.01$ respectively) and 200 μM (both $P < 0.01$).

The second pulse of veratridine resulted in an average aspartate release of $72.8 \pm 3.9\%$ ($n = 6$), and an average second pulse release of glutamate of $72.4 \pm 6.4\%$ ($n = 6$). Veratridine-stimulated release of both aspartate and glutamate release was significantly decreased by **S**(+)-losigamone (Figure 1b), 100 μM (both $P < 0.05$) and 200 μM ($P < 0.01$).

*Effects of **R**(−)-losigamone on amino acid release*

R(−)-losigamone (100, 200 and 400 μM) had little or no effect on potassium or veratridine-stimulated release of aspartate or glutamate (Figure 2a,b).

Neither isomer had any effect on basal release of amino acids. The stimulated release of glutamine, serine, glycine, taurine and GABA was also unaffected by either isomer.

*Effects of **S**(+)-losigamone on spontaneous depolarizations in cortical wedges*

Wedges prepared from DBA/2 mice have been shown to exhibit spontaneous depolarizations when perfused with

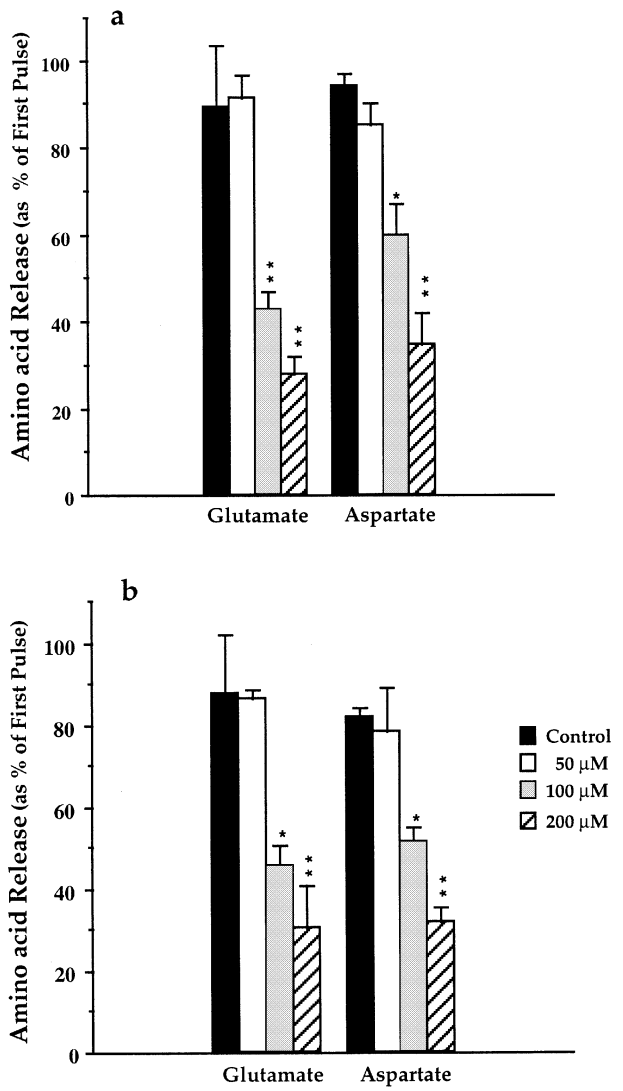

Figure 1 The effect of **S**(+)-losigamone on (a) potassium- (60 mM) and (b) veratridine-induced (20 μM) release of glutamate and aspartate. The results are expressed as the mean ± s.e.mean stimulated release in the second pulse as a percentage of the first pulse. $n = 6-8$ slice preparations. *$P < 0.05$, and **$P < 0.01$.

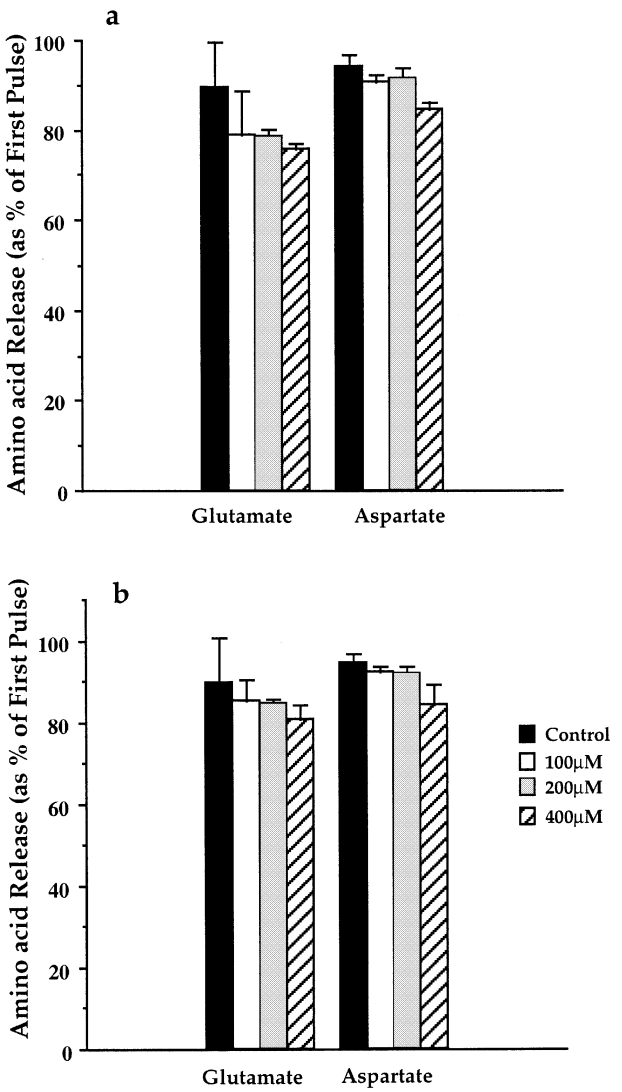

Figure 2 The effect of **R**(−)-losigamone on (a) potassium- (60 mM) and (b) veratridine-induced (20 μM) release of glutamate and aspartate. The results are expressed as the mean ± s.e.mean stimulated release in the second pulse as a percentage of the first pulse. $n = 6-8$ slice preparations.

magnesium-free aCSF. The frequency of these depolarizations was between 5–18 min and were relatively fast in character with rise times of 70–120 ms. The frequency of the depolarizations increased for the first 1–1.5 h of perfusion with magnesium-free aCSF but were stable thereafter for up to 6 h.

S(+)-losigamone perfused for 15 min at 50, 100 and 200 μM produced significant ($P<0.01$) block of magnesium-free aCSF-induced spontaneous depolarizations (Figure 3a) when the number of depolarizations in the control period was compared to those occurring during drug perfusion; no significant block was observed during the third period (i.e. 15 min after the end of drug perfusion).

*Effects of **R**(−)-losigamone on spontaneous depolarizations*

R(−)-losigamone perfused for 15 min at concentrations of 200, 400 and 800 μM produced a significant block of magnesium-free aCSF-induced spontaneous depolarizations during drug perfusion (Figure 3b); 200 μM $P<0.05$; 400 and 800 μM both $P<0.01$).

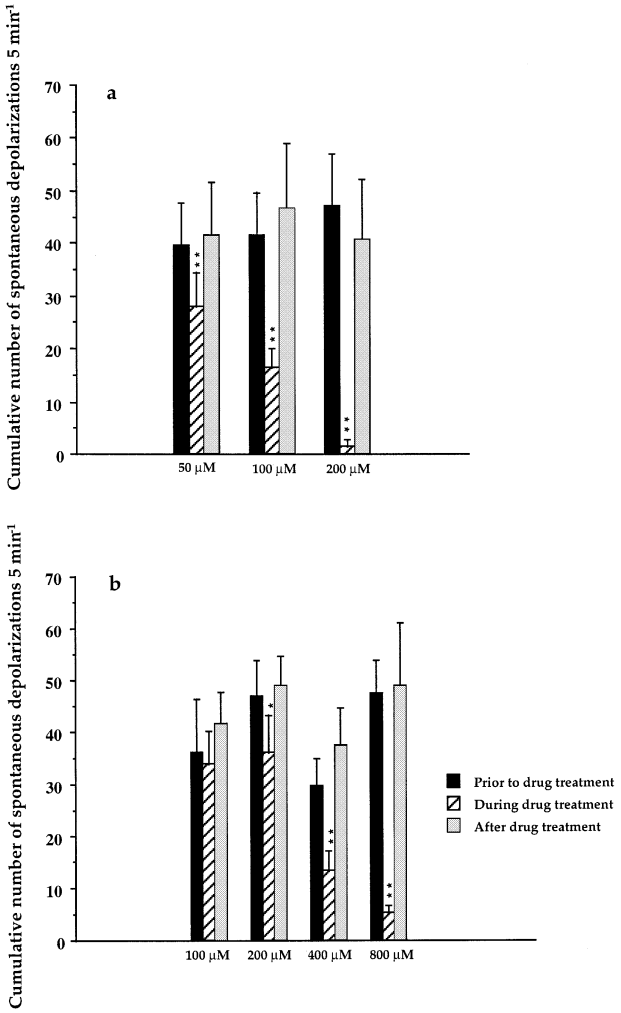

Figure 3 The effects of (a) **S**(+)-losigamone and (b) **R**(−)-losigamone on magnesium-free aCSF-induced spontaneous depolarizations. The results are expressed as mean number of depolarizations per 5 min period (±s.e.mean). $n=4-8$ slice preparations. $^*P<0.05$, $^{**}P<0.01$.

Table 1 Effect of losigamone and its two enantiomers on audiogenic seizures in DBA/2 mice

Treatment	*Dose* mg kg^{-1}	*% Protected*	n
Saline		0	13
S(+)-losigamone	2.5	8	12
	5	50*	12
	10	60**	15
	20	91**	11
R(−)-losigamone	20	0	11
Losigamone	20	27	15

$^*P<0.05$ and $^*P<0.01$ from Saline (Control).

*Effects of **S**(+)-losigamone on audiogenic seizures in DBA/2 mice*

Control mice were treated with sterile saline (0.9%) containing DMSO (0.5%). No significant protection was observed; none of the mice tested being protected from clonic-tonic seizures.

S(+)-losigamone, at all doses tested, had no overt behavioural effects on DBA/2 mice. The compound significantly protected the mice from audiogenic-induced seizures at concentrations of 5, 10 and 20 mg kg^{-1} i.p. (5 mg kg^{-1}, $P<0.05$; 10 and 20 mg kg^{-1} both $P<0.05$; Table 1) when tested 1 h after administration, the end-point being tonic hindlimb extension. Ninety-one per cent of the mice treated with 20 mg kg^{-1} were protected, with 50% of mice still protected at 5 mg kg^{-1}

*Effects of **R**(−)-losigamone and losigamone on audiogenic seizures*

R(−)-losigamone and losigamone (both at 20 mg kg^{-1}) did not produce any statistically significant protection from audiogenic seizures although 27% of the mice were protected with the racemic mixture (Table 1).

Discussion

The results in the present study show that one enantiomer, **S**(+)-losigamone, is effective in protecting against audiogenic seizures in DBA/2 mice and blocking both excitatory amino acid release and spontaneous depolarizations elicited by magnesium-free aCSF. The other enantiomer, **R**(−)-losigamone, is only effective at blocking magnesium-free aCSF-induced spontaneous depolarizations but at considerably higher concentrations than **S**(+)-losigamone.

Results on release showed that veratridine- and potassium-stimulated release of both aspartate and glutamate was specifically and significantly reduced by **S**(+)-losigamone at concentrations of 100 μM and above but **R**(−)-losigamone (100–400 μM) had no effect. The mechanism by which potassium stimulates release is secondary to changes in the transmembrane potential which results in the opening of voltage-sensitive calcium channels leading to exocytosis. The release of neurotransmitters in response to potassium does not involve sodium channels as tetrodotoxin (TTX) is ineffective in reducing this release (Dickie & Davies, 1992). Although losigamone has been shown to block sodium channels (Schmitz *et al.*, 1995) this action is therefore probably not involved in the reduced release seen in these present experiments. The other postulated action of losigamone is through NMDA receptor antagonism as we have previously shown that losigamone inhibited NMDA-induced depolarizations in cortical wedges, and was without effect on α-amino-3-

hydroxy-5-methyl-4-iosxazolapropionic acid (AMPA)-induced depolarizations (Srinivasan *et al*., 1997). NMDA has been shown to stimulate glutamate release from mouse cortical slices (Rowley *et al*., 1993). The glutamate released from the slice probably comes from glutamatergic association or commisural fibres, and the dendrites of these neurones possess NMDA receptors (Huntley *et al*., 1994). If an NMDA antagonist, as is probably the case with **S**(+)-losigamone, prevents activation of these receptors then augmentation of glutamate release by NMDA receptor stimulation will not occur and we have previously shown that the NMDA receptor channel antagonist, dizocilpine, reduced potassium-stimulated release of glutamate (Srinivasan *et al*., 1995). The involvement of NMDA receptors in the mechanism of action of losigamone is at variance with the findings of Stein and co-workers (1991) in that in their study, losigamone was ineffective in antagonizing NMDA-induced convulsions in mice.

Veratridine stimulates the release of neurotransmitters by preventing the inactivation of sodium channels and TTX, through blockade of sodium channels, is effective in preventing this release (Levi *et al*., 1980; Minchin, 1980; Dickie & Davies, 1992). Antiepileptics such as lamotrigine, which have been shown to block veratridine-stimulated release but not potassium-stimulated release, are thought to act by maintaining the inactivation of sodium channels (Leach *et al*., 1986). Work by Schmitz and colleagues (1995) is consistent with the observation that losigamone is a sodium channel blocker as they showed that the drug was effective in reducing sustained repetitive-firing in hippocampal-entorhinal cortical slices, a test which involves the activation of voltage-operated sodium channels. These results would suggest the inhibitory effects of losigamone, and presumably therefore **S**(+)-losigamone, on veratridine-stimulated release of excitatory amino acids may involve sodium channel blockade.

Magnesium ions maintain a natural block of NMDA receptors (*in vivo* and *in vitro*), but when these ions are omitted and magnesium-free aCSF is perfused over cortical wedges spontaneous depolarizations occur. **S**(+)-losigamone was effective in blocking these spontaneous depolarizations which again suggests that it is an NMDA antagonist. Similar results were obtained by Zhang and co-workers (1992) on hippocampal slices perfused with low magnesium aCSF. These observations support the results described on release that in the blockade of potassium-stimulated release of glutamate was probably through NMDA-receptor antagonism.

Audiogenic seizures observed in DBA/2 mice provide a sensitive screening test for potential anticonvulsant drugs. It is not possible to indicate the most appropriate clinical use of drugs screened in this model as, in general, it does not discriminate between the different clinical categories of anticonvulsant drug. It was surprising, however, that **S**(+)-losigamone significantly protected DBA/2 mice from audiogenic seizures whilst the **R**(−)-isomer was without effect at 20 mg kg^{-1}. There was also a significant difference between the animals treated with **S**(+)-losigamone and racemic losigamone. This discrepancy may be due to differences in the metabolism of **S**(+)-losigamone and losigamone. Torcin and co-workers (1996) demonstrated that the major metabolite produced following the incubation of **S**(+)-losigamone with p450 enzymes differed significantly from levels found following incubation with losigamone. It was suggested by the group that **R**(−)-losigamone was competitively inhibiting the production of the metabolite. This observation could account for the significantly differing potencies between **S**(+)-losigamone and racemic losigamone in the audiogenic seizure model. There is no doubt that **S**(+)-losigamone is the more important active constituent of the racemic mixture. It not only possesses the GABA-potentiating properties of the **R**(−)-enantiomer (Chatterjee & Noldner, 1997), but can also suppress overstimulation of the excitatory pathways through reduction of glutamate and aspartate release.

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References

BARNES, S., LEIGHTON, G.E. & DAVIES, J.A. (1988). A novel superfusion system for the measurement of endogenous glutamate release from cerebellar slices. *J. Neurosci. Meth.,* **23,** 57 – 61.

CHATTERJEE, S.S. & NOLDNER, M. (1997). Losigamone: From plant extract to antiepileptic drug. *CNS Drug Rev.,* **3,** 225 – 244.

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 $GABA_A$-receptor system. *Epilepsia,* **36,** 983 – 989.

HAUSER, W.A. & ANNEGERS, J.F. (1993). Epidemiology of epilepsy. In: Laidlaw, J., Richen, A. & Chadwick, D. (eds). *Textbook of epilepsy*. Churchill Livingstone: Edinburgh, 23 – 45.

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

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

KAPETANOVIC, I.M., YONEKAWA, W.D. & KUPFENBERG, 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.

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.

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

LOSCHER, W. (1993). Basic aspects of epilepsy. *Curr. Opin. Neurol. Neurosurg.,* **6,** 223 – 232.

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

RICHENS, A. & PERUCCA, E. (1993). In: *Textbook of epilepsy*. Laidlaw, J., Richens, A. & Chadwick, D. (eds). Churchill Livingstone: Edinburgh, 495 – 559.

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.

SANDER, J.W.A.S. (1998). New drugs for epilepsy. *Curr. Opin. Neurol.,* **11,** 141 – 148.

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 amio acid release from mouse cortex. *Br. J. Pharmacol.,* **116,** 3087–3092.

SRINIVASAN, J., RICHENS, A. & DAVIES, J.A. (1997). The effects of losigamone (AO-33) on electrical activity and excitatory amino acid release in mouse cortical slices. *Br. J. Pharmacol.,* **122,** 1490–1494.

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

STEIN, U., KLESSING, K. & CHATTERJEE, S.S. (1991). Losigamone. In: Pisani, F., Perucca, E., Avanzini, G. & Richens, A. (eds). *New antiepileptic drugs*. (*Epilepsy Res*. suppl. 3). Elsevier: Amsterdam, 129–133.

TORCHIN, C.D., MCNEILLY, P.J., KAPTENOVIC, I.M., STRONG, J.M., HARVEY, J.K. (1996). Stereoselective metabolism of a new anticonvulsant drug candidate, Losigamone, by human liver microsomes. *Drug. Metab. and Disp.,* **24,** 1002–1008.

TURNELL, D.C. & COOPER, J.D.H. (1981). Rapid assay for amino acids in serum or urine by pre-column derivatization and reverse-phase liquid chromatography. *Clin. Chem.,* **28,** 527–531.

ZHANG, C.L., CHATTERJEE, S.S., STEIN, U. & HEINEMANN, U. (1992). Comparison of the effect of losigamone and its isomers on maximal electroshock-induced convulsions in mice and on three different patterns of low-magnesium-induced epileptiform activity in slices of rat temporal cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **345,** 85–92.

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