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The novel antiepileptic drug lacosamide blocks behavioral and brain metabolic manifestations of seizure activity in the 6 Hz psychomotor seizure model

Gary E. Duncan [a,*], Harold Kohn [b]

[a] *Department of Psychiatry, School of Medicine, CB# 7090, 410 Taylor Hall, University of North Carolina, Chapel Hill, NC 27599-7090, USA*

[b] *Division of Medicinal Chemistry and Natural Products, School of Pharmacy, CB# 7360, 210 Beard Hall, University of North Carolina, Chapel Hill, NC 27599-7360, USA*

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Abstract

Brain metabolic activation after 6 Hz electrical stimulation (32 mA, 3 s stimulus duration) was assessed by autoradiographic analysis of ^{14}C-2-deoxyglucose (2-DG) uptake. In addition, effects of the new antiepileptic drug lacosamide were examined on the stimulation-induced metabolic activation. The 6 Hz stimulation via corneal electrodes induced a robust increase 2-DG uptake in cerebral cortical regions, lateral amygdala, and the caudate-putamen. Many other brain regions were not affected by the stimulation, including the hippocampal formation, medial nuclei of the amygdala, thalamus, and hypothalamus. Lacosamide (20 mg/kg) injected i.p. 30 min before application of electrical stimulation antagonized completely the seizure-induced brain metabolic activation but did not affect basal 2-DG uptake. The data provide evidence that lacosamide antagonizes the neural activation induced by an electrical seizure stimulus, without suppressing normal brain metabolic activity.

Keywords: Seizure; 6 Hz; 2-Deoxyglucose; Antiepileptic drug; Lacosamide; Brain metabolism; Cerebral cortex; Hippocampus

1. Introduction

The mainstay of treatment for epileptic disorders has been the long-term and consistent administration of anticonvulsant drugs (Rogawski and Porter, 1997; McNamara, 2001; Aiken and Brown, 2000; Brodie and Dichter, 1996). Unfortunately, current medications are ineffective for approximately one-third of patients with epilepsy (McCorry et al., 2004; Duncan, 2002; Bauer and Reuber, 2003; Mattson et al., 1985). Many continue to have seizures while others experience disturbing side effects (e.g., drowsiness, dizziness, nausea, liver damage) (Pellock and Willmore, 1991). Thus, the need to develop new antiepileptic agents

* Corresponding author. Tel.: +1 919 966 8237; fax: +1 919 966 8237.
E-mail address: gduncan@med.unc.edu (G.E. Duncan).

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with improved efficacy and safety is a continuing priority.

In 1985, we discovered a novel class of anticonvulsant agents, termed functionalized amino acids (FAA) (Cortes et al., 1985). More than 250 FAAs were synthesized and screened in animal model systems (Cortes et al., 1985; Kohn and Conley, 1988; Choi et al., 1996). Where possible, we also compared the anticonvulsant activities for the FAA's (*R*)- and (*S*)-stereoisomers. We found that the anticonvulsant activity resided in the (*R*)-enantiomer. The ratio of the potency of the more active (eutomer) to the less active (distomer) isomer (Lehmann, 1982) ranged from 10 to >22. The lead FAA, lacosamide ((*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide) (Choi et al., 1996), is an emerging drug for the treatment of epilepsy and neuropathic pain and has entered phase III clinical trials in the United States and Europe (Hovinga, 2002, 2003; McCleane et al., 2003).

Preclinical studies documented the anticonvulsant activity of lacosamide. The ED_{50} values for lacosamide in the MES test (Levy et al., 1995) in mice (i.p.) and rats (p.o.) were 4.5 and 3.9 mg/kg, respectively (Choi et al., 1996). When tested against the chemical convulsants, Metrazol (Met) (s.c.), bicuculline (s.c.), and picrotoxin (s.c.), no protection was observed. However, in the sensitive ivMet seizure threshold test, lacosamide elevated the seizure threshold (Bialer et al., 2001). Lacosamide also proved highly effective in the blockage of sound-induced seizures in the genetically susceptible Frings mouse model (ED_{50} = 0.6 mg/kg,) and focal seizures in the hippocampal kindled rat test (ED_{50} = 14 mg/kg) (Bialer et al., 2001). These combined findings differentiated lacosamide's profile from lamotrigine, valproate, carbamazepine, phenytoin, ethosuximide, and other prototypical antiepileptic drugs. Attempts to elucidate the mechanism of action of lacosamide have been unsuccessful. Radioligand displacement binding experiments at nearly 100 potential binding sites, including both ligand- and voltage-gated receptors, have failed to identify a high affinity binding site. Similarly, electrophysiology studies have been disappointing (Hovinga, 2002, 2003).

Important for this study, lacosamide provided excellent protection in mice (i.p.) against seizures (ED_{50} = 10 mg/kg) induced by 6 Hz electrical stimulation (Barton et al., 2001) in the psychomotor assay (Stoehr, T., private communication). A recent investigation suggested that this seizure test serves as a useful model for therapy-resistant limbic seizures (Barton et al., 2001). The 6 Hz stimulation model of partial seizure involves a relatively long stimulus duration (3 s) that produces a seizure characterized behaviorally by forelimb clonus followed by stereotype behaviors, elevation of the tail (Straub tail), and immobility.

The 6 Hz stimulation paradigm is a complementary approach to MES for screening and characterizing antiepileptic drug action, since the two models display different sensitivities to distinct classes of agents. For example, levetiracetam shows efficacy in the 6 Hz (Barton et al., 2001) but not in the MES model (Loscher and Honack, 1993). However, phenytoin and related drugs are not active in the 6 Hz model but are effective in the MES model (Brown et al., 1953). In comparison, lacosamide was active in both the 6 Hz and MES models.

The present study was designed to assess alterations in brain metabolic activity induced in the 6 Hz stimulation model and determine if lacosamide could block the brain metabolic activation associated with the seizure. Autoradiographic assessment of [^{14}C]-2-deoxyglucose (2-DG) uptake was used to provide an index of brain glucose uptake. Since the brain relies almost exclusively on glucose as an energy source, autoradiographic measures of brain glucose uptake provide information on regional brain metabolic activity and function (Sokoloff et al., 1977).

2. Methods

2.1. Drug treatment and electrical stimulation

Mice were injected i.p. with saline or lacosamide (20 mg/kg) 30 min before application of electric current via corneal electrodes or sham application of the electrodes to the cornea. Thus, four treatment groups were examined: saline/sham, n = 9; saline/stimulation, n = 13; lacosamide/sham, n = 7; and lacosamide/stimulation, n = 14. The 20 mg/kg dose of lacosamide was chosen for study based on an ED_{50} value of 10 mg/kg in the 6 Hz model (Stoehr, T., private communication). Parameters described by Barton et al. for corneal stimulation were used in the present study and involved stimulation for 3 s at 6 Hz and 32 mA via a Grass S48 stimulator. For injection of 2-DG, mice

were restrained in a plastic cylinder, their tails warmed to 50 °C with water, and injected via the tail vein with the radiolabeled tracer (1.0 μCi/g body weight, 300 mCi/mmole, American Radiolabeled Chemicals, St. Louis). Mice were then given the active or sham stimulation under hand-restraint approximately 30 s after the injection of 2-DG, returned to their home cage and killed by decapitation 5 min after the 2-DG injection.

2.2. Autoradiographic assessment of 2-DG uptake

The autoradiographic procedures have been described in detail (Duncan et al., 1993, 2002). Brains were removed and frozen in isopentane cooled with liquid nitrogen, stored at −80 °C and sectioned at 10 μm in a cryostat. Autoradiograms were produced in two ways. For quantitative analysis, sections were thaw-mounted onto microscope slides and apposed to Kodak T X-ray film in cassettes for 2 weeks. For high-resolution analysis of brain metabolic patterns, Kodak SR X-ray film was attached to microscope slides with silicone glue and sections were thaw-mounted directly from the microtome knife onto film under safelight conditions. The sections mounted to the film were stored in light-tight desiccator boxes for 4 weeks. Autoradiograms were developed in Kodak D-19 for 10 min (SR film) or 5 min (T film), rinsed in stop bath for 10 s, and fixed in Kodak general purpose fixer for 30 min.

Autoradiograms of brain sections and [^{14}C]-standards (Amersham microscale) were digitized with a high-resolution scanner (Microteck 9800 XL) and quantified using NIH Image Software. Amount of radioactivity in different brain regions of interest were assessed bilaterally in four sections of each mouse. The amount of radioactivity in each brain region for each mouse was determined by reference to the [^{14}C]-standards.

2.3. Statistical analysis

Data of 2-DG uptake for each brain region were analyzed by two-way ANOVAs using GraphPad Prism software. When ANOVAs indicated significant group effects, pair wise comparisons were made with Tukey tests and group differences of $p < .05$ considered statistically significant.

3. Results

3.1. Effects of 6 Hz stimulation on behavior and 2-DG uptake

Electrical stimulation (6 Hz, 32 mA for 3 s) induced behavioral manifestations of seizures and dramatic alterations in 2-DG uptake in the mouse brain. The behavioral expression of seizure activity varied among the mice and included marked forelimb tremor with Straub tail, stereotypic head movements, and immobility with forelimb or vibrissae tremor. Autoradiograms of 2-DG uptake from representative animals are in Fig. 1 and results from quantitative analysis are in Fig. 2. The entire cerebral cortical mantel, lateral nuclei of the amygdala, and caudate-putamen showed marked activation of 2-DG uptake in response to the stimulation. Within the cerebral cortex, the metabolic activation was not uniform and similar "blotchy" regional patterns of increased 2-DG uptake were seen in all animals. Medial nuclei of the amygdala, hippocampus, thalamic and hypothalamic regions were not activated. There were very sharp boundaries between regions activated and not activated by the stimulation.

3.2. Effects of lacosamide on 6 Hz stimulation-induced 2-DG uptake and behavior

Ten of 13 mice pretreated with saline before the stimulation showed clear behavioral signs of seizure activity. In the mice pretreated with lacosamide (20 mg/kg, 30 min prior to stimulation), seizure response was observed in only 1 of the 14 mice tested after stimulation. For the mice showing no behavioral signs of seizure after lacosamide given before application of the stimulation, no increase in 2-DG uptake was apparent. For the one mouse pretreated with lacosamide that showed behavioral signs of seizure activity, there was a unilateral pattern of increased 2-DG uptake. At the lacosamide dose of 20 mg/kg, no significant alteration in 2-DG uptake was apparent in sham-stimulated animals in comparison to the saline controls in any brain region examined. In brain regions that did not show an activation of 2-DG uptake in response to the electrical stimulation, there were no apparent differences between the lacosamide/stimulated and saline/stimulated groups.

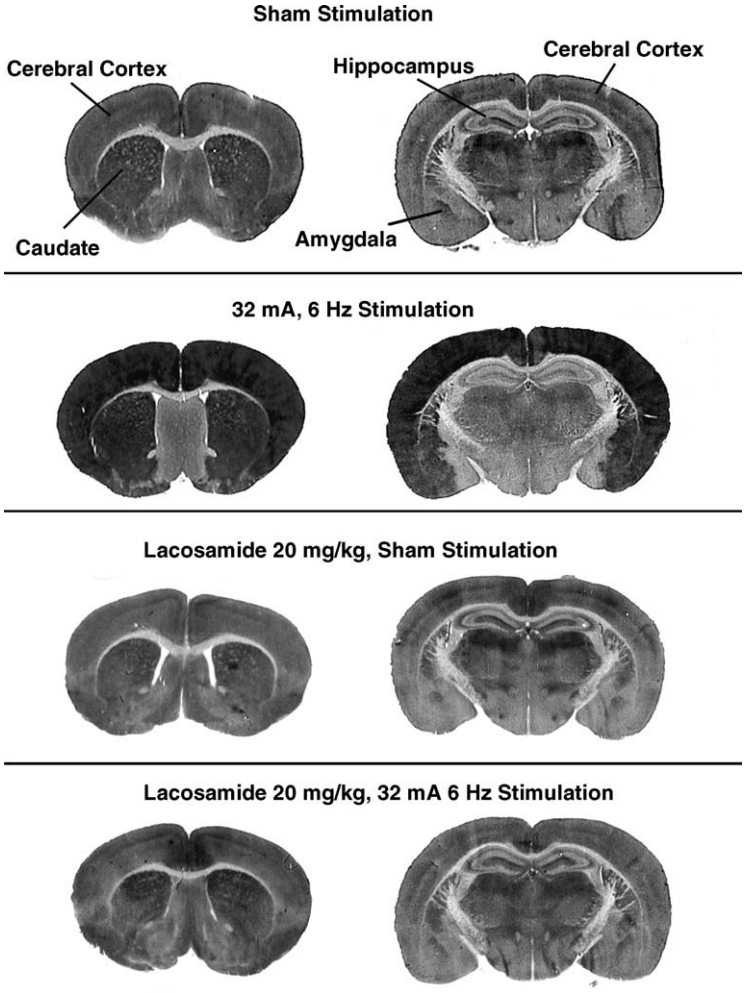

Fig. 1. Autoradiograms of 2-DG uptake showing effects of lacosamide on metabolic activation induced by 6 Hz electrical stimulation. Representative autoradiograms from two different coronal levels are shown for each treatment conditions. Lacosamide (20 mg/kg) or saline was administered 30 min before sham or electrical stimulation (32 mA, 6 Hz for 3 s).

4. Discussion

Brain tissue relies almost exclusively on glucose as an energy source and assessment of regional brain 2-DG uptake provides an index of brain glucose utilization and regional functional activity (Sokoloff et al., 1977). In the present study, we applied autoradiographic techniques to measure regional brain 2-DG uptake in response to 6 Hz electrical stimulation using parameters described by Barton et al. (2001). The stimulation led to marked and selective increases in regional brain 2-DG uptake in the cerebral cortex, caudate-putamen, and lateral amygdaloid nuclei. It is notable that the hippocampal formation was not activated.

Under the stimulation conditions used the entire cerebral cortical mantel was activated, suggesting non-selective activation of different functional regions of the cerebral cortex, such as motor, sensory, and limbic regions. Within the activated cerebral cortical regions, however, the increased 2-DG uptake was not uniform, suggesting some selectivity at a cellular microcircuit level for altered functional activity related to the seizure. The substantial activation of the caudate-putamen observed may have resulted from

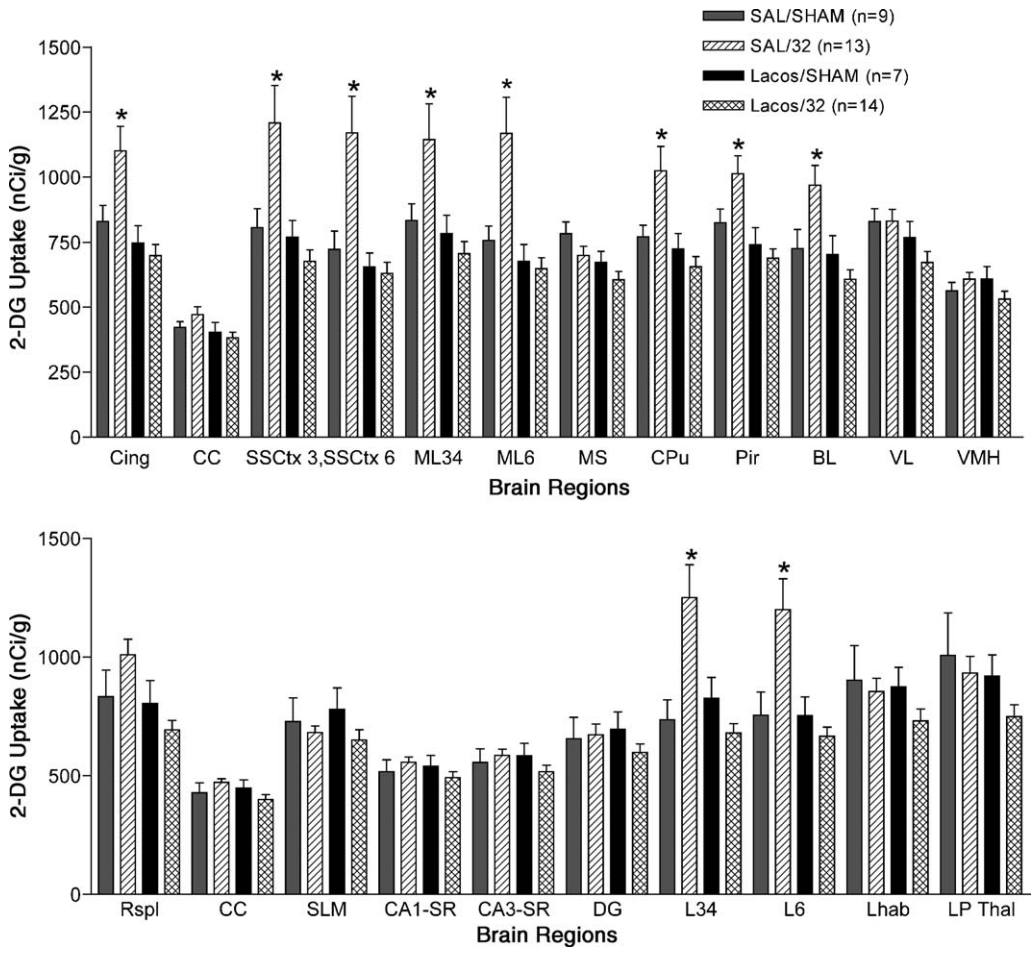

Fig. 2. Effects of lacosamide (20 mg/kg) on 2-DG uptake in mice processed in the 6 Hz seizure paradigm. Lacosamide (Lacos) or saline (SAL) was administered 30 min before sham or electrical stimulation. *Abbreviations*: Cing, cingulate cortex; CC, corpus callosum; SSCtx 3 and 6, jaw region of somatosensory cortex layers 3 and 6; ML 34 and 6, motor cortex layers 3, 4, and 6; MS, medial septum; CPU, caudate-putamen; Pir, piriform cortex; BL, basolateral amygdala; VL, ventral lateral thalamic nucleus; VMH, ventral medial hypothalamus; Rspl, retrosplenial cortex; SLM, stratum lacunosum moleculare of hippocampus; CA1-SR and CA3-SR, stratum radiatum of hippocampus; DG, dentate gyrus; L3, 4, and 6, whisker region of somatosensory cortex; Lhab, lateral habenula; LPthal, lateral posterior thalamic nucleus; $^{*}p < 0.05$ compared to saline-sham.

synaptic activation through cortical–striatal projections. However, further study is required to determine if the activation of the caudate-putamen is due to stimulation of cortical projections to the striatum or to direct effects of the stimulation. Similarly, we cannot determine whether the activation of the lateral amygdala resulted from direct current effects or activation of synaptic efferents. The very sharp boundaries observed between metabolically activated and non-activated regions suggest that specific pathways are involved in the generation and spread of the seizure.

Our findings assessing brain activation by 2-DG uptake agree well with a similar study by Barton et al. (2001) showing increased levels of Fos protein in the cerebral cortex and amygdala, but not in the hippocampus, thalamus, or hypothalamus after 6 Hz stimulation at 32 mA (Barton et al., 2001). Fos is a nuclear protein and transcription factor that is induced by stimuli associated with increased intracellular calcium levels and provides a measure of activation of the cell body of neurons. In contrast, 2-DG uptake reflects a metabolic activity predominately in neuropil

(Duncan et al., 1987; Erecinska and Silver, 1994), which is composed of dendrites, axons and their terminals, and glial cell processes. Increases in 2-DG uptake are related to energy involved in maintaining and re-establishing ionic gradients (Erecinska and Silver, 1994) and in glutamate/glutamine cycling to support glutamate-mediated neurotransmission (Patel et al., 2004). Thus, increased Fos induction and 2-DG uptake involve quite different physiological processes but both approaches indicate similar neuroanatomical patterns of brain activation in the 6 Hz stimulation paradigm.

Pretreatment of mice with lacosamide (20 mg/kg, i.p.) blocked the increased 2-DG uptake in all brain regions induced by 6 Hz stimulation. As previously noted, the dose of lacosamide was chosen based on data indicating an ED_{50} value of 10 mg/kg for protection against 6 Hz seizure induction in behavioral studies. Significantly, lacosamide did not alter the 2-DG uptake in the sham-stimulated animals in any region. These data indicate that lacosamide can suppress seizure-induced brain activation at a dose that has minimal effect on brain metabolism under the sham-stimulation conditions. The antagonism of the metabolic activation induced by the 6 Hz stimulation suggests that lacosamide blocks the generation of the seizure and not just seizure spread.

Differential sensitivity of the drugs in the standard MES model and the 6 Hz model indicate that different neurochemical mechanisms are involved in the initiation and spread of seizures produced by the different types of electrical stimulation. For MES, standard stimulation parameters are 60 Hz stimulation for 0.2 s, whereas in the 6 Hz stimulation model, the duration of the stimulus is much longer (3 s). The original characterization of the 6 Hz model indicated that the model was not wholly predictive of antiepileptic activity for drugs with demonstrated clinical efficacy. Although phenobarbital, phenurone, and mesantoin were active in the 6 Hz model, clinically effective hydantoins, including phenytoin, were not active (Brown et al., 1953). By contrast, first-generation antiepileptic drugs such a phenytoin are active in the MES model (Levy et al., 1995). The inactivity of clinically efficacious antiepileptics in the 6 Hz stimulation model led to an abandonment of the model as a general screening method or new antiepileptics. Recent studies, however, demonstrated that some drugs show weak or no activity and have a non-linear dose–response curves in the MES-test but are active in the 6 Hz paradigm (Barton et al., 2001). One of the most notable examples is levetiracetam. The fact that levetiractetam is effective in refractory human partial epilepsies and active in the 6 Hz model suggests that the 6 Hz model may have utility for identifying drugs with novel properties (Barton et al., 2001). In addition to levetiracetam, antagonists of metabotropic glutamate receptors (mGlu1 and mGlu2/3) (Barton et al., 2003), and GABA-active neurosteroids are active in the 6 Hz model (Kaminski et al., 2004) but show weak or no activity in the MES model (Kokate et al., 1994). Since drugs of widely varying pharmacology are active in the 6 Hz model, it is not possible in the present study to infer the mechanistic pathways for lacosamide action in this seizure model.

The behavioral studies demonstrating lacosamide's activity in both the 6 Hz and the MES models suggest broad potential antiseizure and therapeutic actions for the drug. Indeed, results of recent clinical trials document its therapeutic potential. In a large open-label trial that included 91 patients with uncontrolled partial seizures, addition of oral lacosamide (100–600 mg/day) to the existing AED treatment regimen reduced median seizure frequency by 32% during 4 weeks of maintenance treatment (Sachdeo et al., 2003). In a subsequent placebo-controlled trial in 418 patients with uncontrolled seizures, adjunctive maintenance lacosamide treatment (12 weeks) reduced seizure frequency to a greater extent than placebo. In this latter study, lacosamide at doses of 200, 400, and 600 mg/day resulted in a median seizure frequency reductions of 26, 39, and 40%, respectively, compared with a placebo reduction of 10% (Ben-Menachem et al., 2005). Our studies showed that lacosamide prevents seizure-induced activation at doses that produce minimal effects on normal brain metabolism. The mechanism of action of lacosamide remains unknown and is under investigation.

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