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The novel antiepileptic drug carisbamate (RWJ 333369) is effective in inhibiting spontaneous recurrent seizure discharges and blocking sustained repetitive firing in cultured hippocampal neurons

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Summary This study was initiated to investigate effects of the novel neuromodulator carisbamate (RWJ 333369) in the hippocampal neuronal culture model of status epilepticus and spontaneous epileptiform discharges. Whole-cell current clamp techniques were used to determine the effects of carisbamate on spontaneous recurrent epileptiform discharges (SREDs, in vitro epilepsy), depolarization-induced sustained repetitive firing (SRF) and low Mg^{2+}-induced continuous high frequency spiking (in vitro status epilepticus). This in vitro model is an important tool to study the effects of anticonvulsant drugs (AEDs) on SREDs that occur for the life of the neurons in culture. Carisbamate dose dependently blocked the expression and reoccurrence of SREDs. The ED_{50} value for its antiepileptic effect was $58.75 \pm 2.43\,\mu M$. Inhibition of SRF is considered a common attribute of many AEDs. Carisbamate (100 μM) significantly decreased SRF in hippocampal neurons. All these effects of carisbamate were reversed during a 5 to 30 min drug washout period. When exposed to low Mg^{2+} medium cultured hippocampal neurons exhibit high frequency spiking. This form of in vitro status epilepticus is not effectively blocked by conventional AEDs that are known to be effective in treating status epilepticus in humans. Carisbamate, like phenytoin and phenobarbital, had little or no effect on low Mg^{2+}-induced continuous high frequency spiking. These results characterize the effects of carisbamate in the hippocampal neuronal culture model of epileptiform discharges and suggest that the ability of carisbamate to inhibit depolarization-induced SRF may account in part for some of it's anticonvulsant effect.

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Introduction

Epilepsy is one of the most common neurological disorders affecting approximately 1—2% of population worldwide (Hauser and Hesdorffer, 1990; McNamara, 1999). It is characterized by the occurrence of spontaneous, recurrent unprovoked seizure discharges (Lothman et al., 1991). A seizure is the symptomatic, behavioral manifestation of abnormal, disordered, spontaneous and synchronized, high frequency firing of populations of neurons in the CNS (Delorenzo et al., 2005; Lothman et al., 1991; McNamara, 1999). Epilepsy also has severe socio-economic implications (Murray et al., 1996). Although great advances have been made in the development of newer antiepileptic drugs (AEDs) and surgical interventions for the treatment of epilepsy, approximately 40% of the patients are still refractory to treatment with conventional AED's (Duncan et al., 2006; French, 2007). A sizeable population of epileptic patients deals with the issue of daily management of epilepsy that ultimately affects their quality of life (Cramer et al., 1999b). Thus, there is a growing demand for developing newer medications to treat epilepsy.

Carisbamate or RWJ-333369 (*S*-2-*O*-carbamoyl-1-*o*-chlorophenyl-ethanol) is a novel neuromodulator, initially developed by SK-Biopharmaceuticals and now under development by Johnson & Johnson, Pharmaceutical Research & Development L.L.C. (chemical structure shown in Fig. 1). It has completed phase II and initiated phase III clinical trials for treatment of epilepsy (Bialer et al., 2007). Carisbamate has been evaluated as a novel AED and has demonstrated antiepileptic activity in a variety of in vivo seizure models including hippocampal and corneal kindling (Bialer et al., 2007; Rogawski, 2006) and the GAERS model of absence epilepsy (Nehlig et al., 2005). It also inhibits spontaneous recurrent seizures after kainate induced epilepsy (Grabenstatter and Dudek, 2004) and delays or prevents Li-Pilocarpine (Francois et al., 2005) induced epilepsy. Carisbamate is also anticonvulsant against bicuculline and picrotoxin induced seizures and in pentylenetetrazole and maximal electroshock induced seizure models (Bialer et al., 2007; Rogawski, 2006; White et al., 2006). However, the exact mechanism(s) of action of carisbamate is still unknown. The dicarbamate felbamate has been reported to affect variety of ionic currents including the sodium (White et al., 1992), calcium (Stefani et al., 1996) and NMDA currents (Subramaniam et al., 1995). However, significance of this action for seizure protection is uncertain. Thus it is important to determine mechanisms underlying the antiepileptic effects of carisbamate.

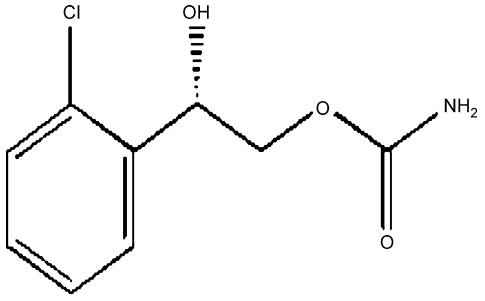

Figure 1 Chemical structure of carisbamate (RWJ 333369). Carisbamate (*S*-2-*O*-carbamoyl-1-*o*-chlorophenyl-ethanol) is a novel neuromodulator under development for the treatment of epilepsy. It has an excellent profile in preclinical models of epilepsy and has demonstrated efficacy and tolerability in a phase II clinical trial. Phase III clinical trials for carisbamate have been initiated for the treatment of epilepsy.

This study was initiated to investigate mechanisms underlying the antiepileptic effects of carisbamate using the hippocampal neuronal culture models of status epilepticus and spontaneous epileptiform discharges (SREDs) (Delorenzo et al., 2005; Deshpande et al., 2007c; Sombati and DeLorenzo, 1995). In addition, we also investigated effects of carisbamate on depolarization-induced sustained repetitive firing in cultured hippocampal neurons. The low Mg^{2+} model utilizes an episode of continuous seizure activity (in vitro status epilepticus) for 3 h. Upon return to normal Mg^{2+} solutions, networks of neurons manifest synchronized SREDs for their life in culture (in vitro model of SREDs). The low Mg^{2+} models of status epilepticus and SREDs in hippocampal neuronal cultures have been routinely used to characterize biochemical, electrophysiological and molecular mechanisms underlying epilepsy in an *in vitro* setting (Churn et al., 2000; Delorenzo et al., 2005; Deshpande et al., 2007c; Pal et al., 2001). These in vitro models also allow for careful control of the neuronal environment and are ideally suited to evaluate the effects of various investigational compounds on electrographic seizure activity.

Materials and methods

All the reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Sodium pyruvate, minimum essential media containing Earle's salts, fetal bovine serum and horse serum were obtained from Gibco-BRL (Invitrogen Corp., Carlsbad, CA). Carisbamate was provided by Johnson & Johnson, Pharmaceutical Research & Development L.L.C., Titusville, NJ, USA.

Hippocampal neuronal culture

All animal use procedures were in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee. Studies were conducted on primary mixed hippocampal neuronal cultures prepared as described previously with slight modifications (Blair et al., 2006; Deshpande et al., 2007b; Sombati and DeLorenzo, 1995). In brief, hippocampal cells were obtained from 2-day postnatal Sprague—Dawley rats (Harlan, Frederick, MD) and plated at a density of 1×10^5 cells/cm^2 onto 35-mm Falcon cell culture dishes (Becton Dickinson and Co., Franklin Lakes, NJ) previously coated with poly-L-lysine (0.05 mg/ml). Glial cultures were maintained at 37 °C in a 5% CO_2/95% air atmosphere and fed thrice weekly with glial feed (minimal essential media with Earle's Salts, 25 mM HEPES, 2 mM L-glutamine, 3 mM glucose, and 10% fetal bovine serum). When confluent, glial beds were treated with 5-μM cytosine arabinoside for 2 days to curtail cell division. On the 13th day in vitro, the media was fully replaced with a 5% horse serum supplemented neuronal feed (composition given below) in preparation for neuronal plating on the following day. At this time, these cultures predominantly consisted of glial cells with few, if any, neurons. On the 14th day in vitro, hippocam-