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THE INHIBITORY ACTIONS BY LACOSAMIDE, A FUNCTIONALIZED AMINO ACID, ON VOLTAGE-GATED NA^+ CURRENTS

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Abstract—The effect of lacosamide (LCS), a functionalized molecule with anti-convulsant properties, on ion channels was investigated, with the aid of patch clamp technology and simulation modeling. In NSC-34 neuronal cells, LCS was found to block voltage-gated Na^+ current (I_{Na}) in a frequency- and concentration-dependent manner. With the two-step voltage protocol, a minimal change in the steady-state inactivation of I_{Na} was found in the presence of LCS. However, with repetitive stimulation, the pulse-to-pulse reduction in peak current was shown to be exponential, with a rate linearly related to both the inter-stimulus interval and the LCS concentration. In addition, the frequency-dependent blocking properties were modeled by considering the drug interaction with a voltage-dependent mixture of Na_V channels harboring either an accessible or an inaccessible binding site. LCS also increased the dimension of inactivation space of Na_V-channel states, thereby producing the adaptive response of neurons to previous firing. LCS (30 μM) had no effects on the non-inactivating component of I_{Na}, while it slightly decreased the amplitude of delayed-rectifier K^+ current. Moreover, LCS suppressed the peak amplitude of I_{Na} in embryonic cortical neurons. In human embryonic kidney (HEK293T) cells which expressed *SCN5A*, LCS attenuated the peak amplitude of I_{Na}, in a concentration-dependent fashion. The unique effects of LCS on Na_V currents presented here may contribute to its *in vivo* modulation of cellular excitability. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: lacosamide, Na^+ current, use dependence, current inactivation, excitability.

INTRODUCTION

Lacosamide (LCS) is a functionalized molecule with anticonvulsant properties which is formerly known as harkoseride, SPM 927 or ADD 234034 (Beyreuther et al., 2007; Krauss et al., 2012). It was effective at suppressing epileptic attacks in different models including generalized or complex partial seizures and status epilepticus (Beyreuther et al., 2007; Stöhr et al., 2007; Höfler and Trinka, 2013; Nunes et al., 2013; Spalletti et al., 2013; Sutter et al., 2013). The mechanism of its action is recognized to be the selective enhancement of slow inactivation in voltage-gated Na^+ (Na_V) channels with no apparent effect on the stabilization of fast inactivation in these channels (Beyreuther et al., 2007; Errington et al., 2008; Krauss et al., 2012).

A recent study showed the ability of LCS to increase the motor thresholds for transcranial magnetic stimulation, further suggesting that this agent could alter neuronal excitability because motor thresholds are recognized to be global measures of corticospinal excitability (Lang et al., 2013). Although a previous study done by Errington et al. (2008) showed that LCS tends to exert selective enhancement in slow inactivation of I_{Na}, the precise molecular mode of action of this agent remains incompletely understood. Interestingly, in contrast to epilepsy, studies showed reduced analgesic effect of LCS and limited effect on I_{Na} inhibition of dorsal root ganglion neurons in a model of peripheral neuropathic pain (Hagenacker et al., 2013), though the differential blocking effect of sensory neuronal I_{Na} by LCS had been established (Sheets et al., 2008). In lines with animal experiments, clinically LCS has limited efficacy in the treatment of peripheral diabetic neuropathy (Hearn et al., 2012).

In addition to the transient I_{Na}, the non-inactivating I_{Na} ($I_{Na(NI)}$), has been identified as having an important role in epilepsy and peripheral neuropathic pain (Stafstrom, 2007). It is known to play an important role in intrinsic bursting behaviors in the epileptic network, responsible for the generation of epileptiform activity (Segal and Douglas, 1997; Huang et al., 2012), as well as maintaining hyperexcitability at dorsal root ganglion neurons in neuropathic pain (Xie et al., 2011). On the other hand, voltage-gated K^+ (K_V) channels play a major role in determining the excitability of neurons. They are responsible for setting the resting potential, repolarizing membranes during action potentials, regulating action potential duration and frequency (Coetzee et al., 1999; Huang et al., 2013). Among them, delayed rectifiers ($I_{K(DR)}$) are

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Abbreviations: FBS, fetal bovine serum; HEK293T, human embryonic kidney cells; *I–V*, current–voltage; LCS, lacosamide; TEA, tetraethylammonium chloride; TTX, tetrodotoxin.

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ubiquitous in neurons. High activation threshold (~−20 mV) and fast deactivation kinetics (< 1 ms at resting potentials) of these K_V channels make them ideal for narrowing spike width and inter-spike interval (Rudy and McBain, 2001). Whether LCS has a distinct effect on these neuronal $I_{Na(NI)}$ and $I_{K(DR)}$ needs to be investigated.

From the viewpoints of safety, although the general safety in LCS pharmacotherapy has been established, demonstrating only some common mild adverse experiences of dizziness, headache, and nausea from large clinical trials (Ben-Menachem et al., 2007; Halász et al., 2009), it has also been reported that the impairment in cardiac conduction in LCS therapy, including electrocardiogram P-R prolongation, sinus node dysfunction, and atrioventricular block (Nizam et al., 2011; Luk et al., 2012; Chinnasami et al., 2013; Malissin et al., 2013), might be related to its pharmacomechanism. Importantly, the ionic effect of LCS outside the central nervous system, especially the Na_v channels (*SCN5A*) on the heart, has not been completely verified.

Our previous studies investigated the transient and late components of I_{Na} observed in culture neurons (Huang et al., 2008, 2012; Wu et al., 2009a). The NSC-34 mouse motor neuron cell line has been recently reported to be a suitable model for investigating the mechanisms of neuronal development, differentiation and stress (Chen et al., 2011; Hsu et al., 2012), and for studying electrophysiological properties (e.g., I_{Na} and $I_{K(DR)}$) (Wu et al., 2012; Huang et al., 2013). Therefore, the objective of this study was to address the question of whether LCS has a specific effect on ionic currents including I_{Na}, $I_{Na(NI)}$ and $I_{K(DR)}$ in NSC-34 neuronal cells, and to determine the use-dependent properties of I_{Na} in the presence of LCS, to evaluate whether LCS influences the dimension of inactivation space of Na_V-channel states, and finally, for further verification of its action target and spectrum, with the aid of molecular biology, we explore whether LCS exerts any effect in embryonic cortical neurons and *SCN5A*-expressing human embryonic kidney (HEK293T) cells.

EXPERIMENTAL PROCEDURES

Experiments were conducted in accordance with the guidelines of the Experimental Ethics Committee of the National Cheng Kung University. Procedures for animal experimentation were reviewed and approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

Drugs and solutions

LCS (*R*-enantiomer of 2-acetamido-*N*-benzyl-3-methoxypropionamide, 2,3-diaminomaleonitrile, Vimpat®; LCS), tefluthrin, tetraethylammonium chloride (TEA), tetrodotoxin (TTX) and cytosine arabinoside were obtained from Sigma–Aldrich (St. Louis, MO, USA), and ranolazine was from Tocris Cookson, Ltd. (Bristol, UK). All culture media, fetal bovine serum (FBS), L-glutamine, trypsin/EDTA, and penicillin-streptomycin were obtained from Invitrogen (Carlsbad, CA, USA). All other chemicals, including $CdCl_2$, CsCl and CsOH, were commercially available and of reagent grade.

The composition of normal Tyrode's solution was as follows (in mM): NaCl 136.5, KCl, 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose, 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). To record membrane potential or K^+ currents, the patch pipette was filled with a solution (in mM): KCl 140, $MgCl_2$ 1, Na_2ATP 3, Na_2GTP 0.1, EGTA 0.1, and HEPES-KOH buffer 5 (pH 7.2). To measure I_{Na}, K^+ ions inside the pipette solution were replaced with equimolar Cs^+ ions and the pH was adjusted to 7.2 with CsOH, and bath solution contained 10 mM TEA.

Cell preparations

NSC-34 neuronal cells were originally produced by fusion of motor neuron-enriched, embryonic mouse spinal cords with mouse neuroblastoma (Hsu et al., 2012; Wu et al., 2012). They were routinely maintained in a 1:1 mixture of DMEM and Ham's F12 medium supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin. Before confluence, cells were grown in 1:1 DMEM plus Ham's F12 medium supplemented with 1% FBS. HEK293T cells were obtained from the American Type Culture Collection ([CRL-11268]; Manassas, VA, USA). They were grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1% penicillin-streptomycin. Cultures were commonly incubated at 37 °C in a humidified environment of 5% CO_2/95% air. For transfection of HEK293T cells, cells at a number of 2–6 × 10^5 were commonly seeded on the 6-cm culture dish for 24 h before transfection.

Transfection

The $Na_V1.5$ DNA construct (*SCN5A*; GenBank™ accession number M77235) was subcloned into a modified pSP64T vector. This plasmid pSP64T-WT-SCN5A was a kind gift from Dr. Alfred L. George (Vanderbilt University Medical Center, Nashville, TN, USA) and Dr. Ru-Chi Shieh (Institute of Biomedical Sciences, Academia Sinica, Taipei City, Taiwan). For transient expression, the plasmid was transfected into HEK293T cells with the use of PEI reagents (ExGen 500; MBI Fermentas, Hanover, MD, USA) (Wu et al., 2009b). When the plasmid was diluted in 150 mM NaCl, it was mixed with PEI and incubated for 10 min at room temperature. After centrifugation at 280*g* for 5 min, cells were incubated at 37 °C for an additional 48 h. The *SCN5A* expression was determined by immunofluorescence staining or electrophysiological measurements.

Isolation of embryonic cortical neurons

Neuronal cultures were prepared from cerebral cortices of embryonic day 15–17 Sprague–Dawley rats (Lin et al., 2008; Pacifici and Peruzzi, 2012). Donor animals were humanely killed, and embryos were collected by Caesarian section. Cortices were minced with a razor blade and dissociated by trituration through the narrowed bore of a fire-polished Pasteur pipette. Isolated cells were plated onto poly(D-lysine)-coated glass shards at densities of