Neuroscience 238 (2013) 59–70

CHARACTERIZATION OF L-TYPE VOLTAGE-GATED CA^{2+} CHANNEL EXPRESSION AND FUNCTION IN DEVELOPING CA3 PYRAMIDAL NEURONS

R. A. MORTON, M. S. NORLIN, C. C. VOLLMER AND C. F. VALENZUELA*

Department of Neurosciences, School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, USA

Abstract—Voltage-gated calcium channels (VGCCs) play a major role during the development of the central nervous system (CNS). Ca^{2+} influx via VGCCs regulates axonal growth and neuronal migration as well as synaptic plasticity. Specifically, L-type VGCCs have been well characterized to be involved in the formation and refinement of the connections within the CA3 region of the hippocampus. The majority of the growth, formation, and refinement in the CNS occurs during the third trimester of human pregnancy. An equivalent developmental time period in rodents occurs during the first 2 weeks of post-natal life, and the expression pattern of L-type VGCCs during this time period has not been well characterized. In this study, we show that $Ca_v1.2$ channels are more highly expressed during this developmental period compared to adolescence (post-natal day 30) and that L-type VGCCs significantly contribute to the overall Ca^{2+} currents. These findings suggest that L-type VGCCs are functionally expressed during the crucial developmental period.

Key words: hippocampus, calcium, calcium channels, postnatal, development.

INTRODUCTION

In the central nervous system (CNS), Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) is essential for a number of processes, including vesicular release of neurotransmitters, intracellular signaling pathways, gene expression, and synaptic plasticity (Turner et al., 2011). Furthermore, VGCCs are tightly coupled to and modulate other ion channels as well as G-protein-coupled receptors (Turner et al., 2011). VGCCs are comprised of a pore-forming α subunit and auxiliary β, γ, and δ subunits (Catterall, 2011). VGCCs are subdivided into five different types: L, N, P/Q, R, and T. In this study, we focused on L-type VGCCs, which can include one of four α-subunit variants: $Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$ or $Ca_v1.4$ (Catterall, 2011). Neurons within the CNS predominantly express $Ca_v1.2$ and $Ca_v1.3$ in an overlapping pattern that includes both somatic and dendritic compartments (Zuccotti et al., 2011). VGCCs containing $Ca_v1.2$ display the typical characteristics of L-type channels, including high-voltage activation, slow activation kinetics, and high sensitivity to dihydropyridines (Lipscombe et al., 2004). In contrast, recombinant studies show that $Ca_v1.3$ channels have a lower voltage activation threshold, faster activation kinetics and are less sensitive to dihydropyridines (Lipscombe et al., 2004).

Alterations in L-type VGCCs containing $Ca_v1.2$ or $Ca_v1.3$ have been linked to neurodevelopmental disorders. A missense gain-of-function mutation in $Ca_v1.2$ (G406R) increases membrane expression and substantially decreases voltage-dependent inactivation, leading to a multisystem disorder known as Timothy syndrome (Splawski et al., 2004). The neuropsychological alterations linked to this syndrome involve language deficits and impairments in social skills that have led to the diagnosis of autism-spectrum disorder in many of these patients (Liao and Soong, 2010). Mice deficient in $Ca_v1.3$ exhibit congenital deafness and neuronal circuit abnormalities that are particularly pronounced in brainstem auditory centers (Platzer et al., 2000; Hirtz et al., 2011). $Ca_v1.3$ knockout mice also display deficits in the consolidation of contextual fear conditioning suggesting functional alterations in the neurons of the hippocampus and basolateral amygdala (McKinney and Murphy, 2006; McKinney et al., 2009; Gamelli et al., 2011). In addition, levels of serotonin, glutamate, GABA, and taurine were shown to be elevated in the striatum of $Ca_v1.3$ knockout mice (Sagala et al., 2012). Although these studies clearly indicate that L-type VGCCs containing $Ca_v1.2$ and $Ca_v1.3$ subunits play an important role in CNS development, the precise mechanisms responsible for the alterations in neuronal circuit development produced by L-type VGCC dysfunction are not fully understood.

The 3rd trimester of human development is a period of significant brain development, gyrification (cortical folding), and synaptogenesis (formation of synapses) (Lodygensky et al., 2010). The equivalent neuronal developmental period in rodents is during the

*Corresponding author. Address: Department of Neurosciences, MSC08 4740, 1 University of New Mexico, Albuquerque, NM 87131, USA. Tel: +1-505-272-3128; fax: +1-505-272-8082.
E-mail address: fvalenzuela@salud.unm.edu (C. F. Valenzuela).

Abbreviations: ACSF, artificial cerebral spinal fluid; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DAPI, 4′,6-diamidino-2-phenylindole; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; GDP, giant depolarizing potential; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; P, postnatal day; VGCC, voltage-gated calcium channel.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.02.008

first 2 weeks of post-natal life (Cudd, 2005; Lodygensky et al., 2010). L-type VGCCs have been shown to be involved in several processes required for the formation and refinement of neuronal circuits. Prior to synapse formation, Ca^{2+} influx via L-type VGCCs regulates axonal growth and neuronal migration (Tang et al., 2003; Hutchins and Kalil, 2008; Takahashi and Magee, 2009). Subsequently, L-type VGCCs participate in the formation and refinement of synaptic connections, which has been particularly well-characterized in the CA3 hippocampal region. This hippocampal region integrates inputs from the dentate gyrus, entorhinal cortex, and neighboring CA3 pyramidal neurons via mossy fibers, perforant path, and recurrent collateral pathways, respectively. CA3 pyramidal neurons also receive inhibitory connections from local interneurons that play a role of feed-forward inhibition (Lawrence and McBain, 2003). During the 3rd trimester equivalent, L-type VGCC-dependent retrograde release of brain-derived neurotrophic factor (BDNF) is thought to contribute to the stabilization of interneuron-CA3 pyramidal neuron synapses via a long-term potentiation-like mechanism (Gubellini et al., 2005; Kuczewski et al., 2008a,b). L-type VGCC/BDNF-dependent plasticity has been shown to play a similar role in mossy fiber-CA3 pyramidal neuron synapse maturation (Kasyanov et al., 2004; Spitzer et al., 2004; Sivakumaran et al., 2009). L-type VGCC/BDNF-dependent plasticity of GABAergic transmission at CA3 pyramidal neurons has been shown to be inhibited by alcohol exposure during the 3rd trimester equivalent, a deficit that may contribute to the hippocampal dysfunction that characterizes fetal alcohol spectrum disorders (Zucca and Valenzuela, 2010). Despite the importance of L-type VGCCs in the formation of CA3 hippocampal neuronal circuits, the developmental expression patterns and function of these channels have not been systematically characterized in this region.

Here, we investigated the developmental expression and distribution of $Ca_v1.2$ and $Ca_v1.3$ subunits in the CA3 hippocampal region using Western immunoblotting and immunohistochemistry. Using whole-cell voltage-clamp slice electrophysiology and Ca^{2+} imaging, we assessed the function of L-type VGCCs in developing CA3 pyramidal neurons. We show that $Ca_v1.2$ expression transiently peaks during the 3rd trimester equivalent compared to adolescence (post-natal day – P30), whereas, $Ca_v1.3$ is expressed at lower levels, but remains relatively constant, throughout development and adolescence. Functionally, 30–40% of total VGCC currents in CA3 pyramidal neurons were mediated by L-type channels between P4 and P15. L-type VGCC-mediated Ca^{2+} transients could be detected in both dendrites and soma of developing CA3 pyramidal neurons.

EXPERIMENTAL PROCEDURES

Animals and slice preparation

The University of New Mexico Health Sciences Center Institutional Care and Use Committee approved all animal procedures. Pregnant Sprague–Dawley dams between gestational day 12 and 17 were received from the Harlan Laboratories (Indianapolis, IN, USA). Both male and female pups were used for all experiments except for the P30 time points when only male offspring were used. Animals were heavily anesthetized with ketamine followed by decapitation. Brain tissue was removed and incubated for 2–4 min in oxygenated ice cold cutting solution (in mM): sucrose, 220; KCl, 2; NaH_2PO_4, 1.3; $NaHCO_3$, 26; $MgSO_4$, 12; $CaCl_2$, 0.2; glucose, 10; ketamine, 1 mg/ml. Coronal slices were generated using a vibrating slicer (1000 Plus Vibratome, Leica, Bannockburn, IL, USA) at a thickness of 300 μm. Slices were incubated in oxygenated artificial cerebral spinal fluid (ACSF) (in mM): NaCl, 125; KCl, 2; NaH_2PO_4, 1.3; $NaCO_3$, 26; glucose, 10, $CaCl_2$, 2; $MgSO_4$, 10 at 35 °C for 40 min and allowed to recover at room temperature (21–22 °C) for at least 30 min prior to recording or dissection. All chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA) unless specified.

Electrophysiology recordings

Slices were maintained in ACSF during recording at approximately 32 °C. CA3 pyramidal neurons were identified by morphology under video monitoring of infra-red Differential Interference Contrast Microscopy using an BX51WI upright microscope (Olympus, Center Valley, PA, USA) and a LUMPlan FI/IR 40× water immersion lens 0.8 N.A. Recording electrodes were pulled using a DMZ-Universal Puller (Zeitz Instruments, Martinsreid, Germany) resulting in resistances between 2 and 5 MΩ. Electrodes were filled with internal solution containing the following (in mM): CsCl, 135; MgCl, 4; HEPES, 10; EGTA, 10; MgATP, 4; NaGTP, 0.3. Internal solution pH was adjusted to 7.2 with CsOH. Recordings were obtained with a Multiclamp 700B amplifier connected to a Digidata 1440A and data were acquired with pClamp 10 software (Molecular Devices Sunnyvale, CA, USA). CA3 pyramidal neurons were filled using regular recording electrodes containing in mM: K-gluconate, 135; NaCl, 8; $MgCl_2$, 1; HEPES, 10 (pH 7.2 with KOH); MgATP, 2; EGTA, 0.05, and Alexa-488 hydrazide, 1 (Invitrogen, Grand Island, NY, USA). In the whole-cell configuration, we compensated for membrane capacitance and 70% of series resistance. VGCC currents were isolated with 1 μM TTX (Tocris, Bristol, United Kingdom), 10 μM GABazine (Tocris), 50 μM DL-APV (Tocris), 10 mM TEA (Sigma), and 1 mM kynurenic acid (Sigma).

Calcium imaging

In the whole-cell patch-clamp configuration, CA3 pyramidal neurons were filled with the CsCl internal solution (described above) supplemented with Bis-Fura-2 hexapotassium salt (150 μM) (Invitrogen). Neurons were allowed to dialyze for 20 min prior to Ca^{2+} imaging. Ca^{2+} transients were acquired using a Polychrom V imaging system from TILL photonics (Grafelfig, Germany) equipped with a monochromator and an optical fiber to deliver light to the microscope. Within the imaging path a 500-nm dichroic mirror and emission filter 525/50 nm were used to image the fluorescence of Fura-2 at both 350 nm and 380 nm. Data acquisition and analysis were performed with TILLvisION imaging software version 4.5.41 (TILL Photonics). Images were acquired at 350 nm and 380 nm at approximately 4 Hz. Ca^{2+} transients were measured at the soma and dendrites (approximately 30–60 μm from the soma). Three Ca^{2+} transients were induced and averaged together with each transient normalized to the pre-depolarization baseline fluorescence. Fluorescence intensities at 350 nm were divided by the fluorescence at 380 nm with background subtracted. Ratiometric images were used to measure the Ca^{2+} transients in both the soma and dendritic compartments.