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MICE LACKING THE TRANSCRIPTIONAL COACTIVATOR PGC-1α EXHIBIT ALTERATIONS IN INHIBITORY SYNAPTIC TRANSMISSION IN THE MOTOR CORTEX

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Abstract—Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a transcriptional coactivator known to regulate gene programs in a cell-specific manner in energy-demanding tissues, and its dysfunction has been implicated in numerous neurological and psychiatric disorders. Previous work from the Cowell laboratory indicates that PGC-1α is concentrated in inhibitory interneurons and is required for the expression of the calcium buffer parvalbumin (PV) in the cortex; however, the impact of PGC-1α deficiency on inhibitory neurotransmission in the motor cortex is not known. Here, we show that mice lacking PGC-1α exhibit increased amplitudes and decreased frequency of spontaneous inhibitory postsynaptic currents in layer V pyramidal neurons. Upon repetitive train stimulation at the gamma frequency, decreased GABA release is observed. Furthermore, PV-positive interneurons in PGC-1α −/− mice display reductions in intrinsic excitability and excitatory input without changes in gross interneuron morphology. Taken together, these data show that PGC-1α is required for normal inhibitory neurotransmission and cortical PV-positive interneuron function. Given the pronounced motor dysfunction in PGC-1α −/− mice and the essential role of PV-positive interneurons in maintenance of cortical excitatory:inhibitory balance, it is possible that deficiencies in PGC-1α expression could contribute to cortical hyperexcitability and motor abnormalities in multiple neurological disorders.

Key words: peroxisome proliferator-activated receptor gamma coactivator 1α, parvalbumin, inhibitory neurotransmission, motor cortex, interneuron.

INTRODUCTION

Peroxisome proliferated-activated receptor γ coactivator 1α (PGC-1α) is a transcriptional coactivator which, by interacting with different transcription factors, initiates cell and tissue-specific gene programs. Since the discovery of PGC-1α in 1998 (Puigserver et al., 1998), many studies have suggested that a reduction in its levels and/or activity plays a role in neurological disorders including Parkinson Disease (Zheng et al., 2010), Alzheimer Disease (Qin et al., 2009; Sheng et al., 2012), Huntington Disease (Cui et al., 2006; Taherzadeh-Fard et al., 2009; Chaturvedi et al., 2010), schizophrenia (Christoforou et al., 2007; Jiang et al., 2013b), anxiety disorders (Hettema et al., 2011) and multiple sclerosis (Witte et al., 2013). Studies with whole body and neuron-specific PGC-1α −/− mice indicate that PGC-1α is required for the expression of a subset of metabolic and neuronal transcripts (Lin et al., 2004; Lucas et al., 2010, 2012; Ma et al., 2010), but the physiological consequences of these transcriptional changes are not clear. Elucidating the impact of PGC-1α deficiency on neuronal function will give us insight into its contribution to neuronal dysfunction in various disorders.

The PGC-1α protein is highly concentrated in GABAergic cell populations throughout the brain (Cowell et al., 2007; Jiang et al., 2013b), and PGC-1α −/− mice exhibit deficiencies in the expression of the calcium buffer protein parvalbumin (PV) in forebrain regions including cortex, hippocampus, and striatum (Lucas et al., 2010). In these regions, PV is expressed by a subset of GABAergic interneurons that exhibit fast-spiking (FS) and non-adapting properties (Kawaguchi, 1993; Kawaguchi and Kondo, 2002; Tepper and Bolam, 2004) and entrain local pyramidal neurons to generate gamma oscillations

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Abbreviations: ACSF, artificial cerebral spinal fluid; AHP, afterhyperpolarization; ANOVA, analysis of variance; AP, action potential; CCK, cholecystokinin; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DL-APV, DL-2-amino-5-phosphonoovaleric acid; EGFP, enhanced green fluorescent protein; EGTA, ethylene glycol tetraacetic acid; EPSC, excitatory postsynaptic current; FS, fast-spiking; GAD, glutamic acid decarboxylase; HEPES, hydroxyethyl piperazineethanesulfonic acid; IPSC, inhibitory postsynaptic current; NMDA, N-methyl-D-aspartate; P, postnatal day; PBS, phosphate buffered saline; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PV, parvalbumin; TRITC, tetramethylrhodamine; TTX, tetrodotoxin.

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(Wang and Buzsaki, 1996; Bartos et al., 2002; Vreugdenhil et al., 2003; Sohal et al., 2009). Interestingly, mice lacking PGC-1α show pronounced motor abnormalities and decreased PV protein expression in the motor cortex by 4 weeks of age (Lucas et al., 2012), suggesting that the motor cortex may be particularly dependent on PGC-1α for proper function. Previous investigations of inhibitory neurotransmission in the hippocampus of PGC-1α −/− mice (Lucas et al., 2010) suggest that inhibition is enhanced in this region, similar to what is observed in PV −/− mice (Vreugdenhil et al., 2003). However, it is possible that inhibition in the cortex is affected differentially by a lack of PGC-1α; it is therefore important to evaluate the impact of PGC-1α deficiency in the cortex, with relevance for disorders in which cortical PGC-1α deficits have been reported, including Parkinson Disease (Zheng et al., 2010) and Alzheimer Disease (Qin et al., 2009).

In light of the deficiency in PV expression in the cortex of PGC-1α −/− mice and the profound motor dysfunction in these animals, we sought to determine the physiological impact of PGC-1α deletion on inhibitory neurotransmission in the motor cortex. We hypothesized that mice lacking PGC-1α would exhibit altered inhibitory transmission onto cortical pyramidal neurons and that PV+ interneurons would be especially affected. In order to investigate the potential role of PGC-1α in cortical inhibitory neurotransmission, we utilized motor cortex acute slices from a PGC-1α −/− mouse model (Lin et al., 2004). Our data show that, in contrast to results from the PGC-1α −/− hippocampus, a loss of PGC-1α leads to alterations in basal GABA release in the cortex, concurrent with reduced GABA release upon gamma frequency stimulation. Furthermore, in PGC-1α −/− mice expressing enhanced green fluorescent protein (EGFP) specifically in PV+ cells, we found that FS interneurons have a reduced firing rate in response to current injections, suggesting that PGC-1α functions in a cell-autonomous manner to regulate interneuron excitability. Additionally, PV+ cells showed reduced synaptic excitatory activity, suggesting that PV+ cells could be less active in the motor cortex of PGC-1α −/− mice. Taken together these data suggest that reductions in PGC-1α expression are associated with deficiencies in inhibitory neurotransmission and synaptic function in the cortex. These results have implications for understanding the impact of PGC-1α alterations on cortical network signaling in disease, as synchronization of firing by PV+ interneurons is critical for normal cortical output and higher cognitive processing (Sohal et al., 2009).

EXPERIMENTAL PROCEDURES

Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. PGC-1α −/− mice (generous gift of Jiandie Lin, University of Michigan, (Lin et al., 2004)) were maintained on a C57BL/6J genetic background and housed two to five in a cage at 26 ± 2 °C room temperature with food and water *ad libitum*. All experiments were conducted with 4-week-old male and female PGC-1α +/+ and −/− littermates generated by breeding PGC-1α +/− mice.

For pyramidal neuron recordings, PGC-1α +/+ and −/− mice were used. For targeted interneuron recordings, mice from the PGC-1α −/− line were crossed with mice expressing EGFP under the control of the glutamic acid decarboxylase (GAD67) promoter (G42 line; JAX#7677) to generate EGFP-positive PGC-1α +/+ and EGFP-positive PGC-1α −/− littermates. This mouse line was chosen for two reasons: (1) EGFP is only expressed in the PV+ subset of inhibitory interneurons, primarily in cortex (Chattopadhyaya et al., 2004; Bartley et al., 2008) and (2) EGFP expression is not dependent on the activity of the PV promoter (which would be expected to be reduced in the absence of PGC-1α). All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* adopted by the U.S. National Institutes of Health.

For pyramidal neuron recordings

Whole-cell recordings. Mice aged postnatal day (P) 27 to P33 were anesthetized with isoflourane and then decapitated. Brains were placed in ice-cold artificial cerebral spinal fluid (ACSF) containing the following (in mM): 125 NaCl, 3.5 KCl, 0.5 $CaCl_2$, 3.5 $MgCl_2$, 26 $NaHCO_3$ and 10 D-glucose. The ACSF was bubbled with 95% O_2/5% CO_2. Coronal brain slices (300-μm thick) containing motor cortex were cut using a Vibratome (Ted Pella, Inc., Riverside, CA, USA). The slices were kept for 30 min at 37 ± 1 °C and then stored at room temperature (22 ± 1 °C). Slices were perfused continuously with oxygenated recording ACSF containing the following (in mM): 125 NaCl, 3.5 KCl, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 26 $NaHCO_3$ and 10 D-glucose at room temperature. Whole-cell patch clamp recordings were acquired from visually identified pyramidal neurons in layer five of the motor cortex. Position in cortex was verified through inclusion of 0.4% biocytin in the internal solution followed by streptavidin staining (Invitrogen, Carlsbad, California, US, s32355). Recordings were conducted on a Zeiss AxioExaminer microscope (Carl Zeiss, Thornwood, NY, USA). Cells were voltage clamped at −70 mV, using internal solution containing the following (in mM): 129 CsCl, 2 MgATP, 10 EGTA, 10 HEPES, 0.2 GTP and 2 QX-314, pH 7.2. Pipette tip resistance was 2–5 M. Voltage clamp recordings were obtained using a PC505A amplifier (Warner Instruments, Hamden, CT, USA) controlled by Clampex 8.0 software via a Digidata 1322A interface (Molecular Devices, Union City, CA, USA), filtered at 5 kHz and digitized at 10 kHz. Input resistance and series resistance were monitored by applying a 10-mV voltage step. Spontaneous IPSCs (sIPSCs) and evoked IPSCs were pharmacologically isolated with CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) (10 μM) and DL-APV (DL-2-amino-5-phosphonoovaleric acid) (50 μM). Miniature IPSCs (mIPSCs) were recorded, on a separate cohort of animals, in the presence of CNQX, D-APV, and TTX (tetrodotoxin) (1 μM).