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Mechanism underlying unaltered cortical inhibitory synaptic transmission in contrast with enhanced excitatory transmission in $Ca_V2.1$ knockin migraine mice

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ABSTRACT

Familial hemiplegic migraine type 1 (FHM1), a monogenic subtype of migraine with aura, is caused by gain-of-function mutations in $Ca_V2.1$ (P/Q-type) calcium channels. In FHM1 knockin mice, excitatory neurotransmission at cortical pyramidal cell synapses is enhanced, but inhibitory neurotransmission at connected pairs of fast-spiking (FS) interneurons and pyramidal cells is unaltered, despite being initiated by $Ca_V2.1$ channels. The mechanism underlying the unaltered GABA release at cortical FS interneuron synapses remains unknown. Here, we show that the FHM1 R192Q mutation does not affect inhibitory transmission at autapses of cortical FS and other types of multipolar interneurons in microculture from R192Q knockin mice, and investigate the underlying mechanisms. Lowering the extracellular $[Ca^{2+}]$ did not reveal gain-of-function of evoked transmission neither in control nor after prolongation of the action potential (AP) with tetraethylammonium, indicating unaltered AP-evoked presynaptic Ca influx at inhibitory autapses in FHM1 KI mice. Neither saturation of the presynaptic calcium sensor nor short duration of the AP can explain the unaltered inhibitory transmission in the mutant mice. Recordings of the P/Q-type calcium current in multipolar interneurons in microculture revealed that the current density and the gating properties of the $Ca_V2.1$ channels expressed in these interneurons are barely affected by the FHM1 mutation, in contrast with the enhanced current density and left-shifted activation gating of mutant $Ca_V2.1$ channels in cortical pyramidal cells. Our findings suggest that expression of specific $Ca_V2.1$ channels differentially sensitive to modulation by FHM1 mutations in inhibitory and excitatory cortical neurons underlies the gain-of-function of excitatory but unaltered inhibitory synaptic transmission and the likely consequent dysregulation of the cortical excitatory–inhibitory balance in FHM1.

Introduction

Voltage-gated P/Q-type calcium channels ($Ca_V2.1$) play a prominent role in controlling neurotransmitter release at brain excitatory and inhibitory synapses (Pietrobon, 2005, 2010). Mutations in the gene encoding the $Ca_V2.1\alpha1$ subunit cause several neurological disorders including familial hemiplegic migraine type 1 (FHM1), a rare monogenic form of migraine with aura (Ophoff et al., 1996; Pietrobon, 2010).

Migraine is a common disabling disorder caused by a primary brain dysfunction that leads to episodic activation of the trigeminovascular pain pathway and headache (Pietrobon and Moskowitz, 2013). There is increasing evidence that the headache mechanisms can be triggered by cortical spreading depression (CSD), the phenomenon underlying migraine aura (Pietrobon and Moskowitz, 2013). The molecular and cellular mechanisms of the primary brain dysfunction(s) leading to susceptibility to CSD and to the migraine attack remain a major open issue. Important insights into these mechanisms have been obtained from the analysis of functional consequences of FHM1 mutations, revealing that these mutations produce: (i) gain-of-function of human recombinant and native neuronal mouse $Ca_V2.1$ channels, mainly due to channel activation to lower voltages and increased channel open probability; and (ii) facilitation of induction and propagation of experimental CSD, due to increased glutamate release at cortical pyramidal cell synapses [Pietrobon, 2010; Pietrobon and Moskowitz, 2013) and references therein]. In striking contrast with enhanced glutamatergic neurotransmission, the inhibitory neurotransmission at connected pairs of layer 2/3 fast-spiking (FS) interneurons and pyramidal cells was unaltered

Abbreviations: AHP, after-hyperpolarization; AP, action potential; AP_{hw}, action potential half width; BME, basal Eagle's medium; BSA, bovine serum albumin; CGRP, calcitonin gene-related peptide; CSD, cortical spreading depression; DIV, days in vitro; FHM1, familial hemiplegic migraine type 1; FS, fast-spiking; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; 5HT3aR, 5-hydroxytryptamine 3a receptor; KI, knockin; LJP, liquid junction potential; PBS, phosphate buffered saline; PV, parvalbumin; siRNA, small interfering ribonucleic acid; SNAP25, synaptosomal-associated protein of 25 kDa; SOM, somatostatin; VIP, vasoactive intestinal peptide.

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in FHM1 knockin (KI) mice, despite being initiated by $Ca_V2.1$ channels (Tottene et al., 2009). The differential effect of FHM1 mutations at excitatory and inhibitory synapses suggests altered regulation of the cortical excitatory–inhibitory balance in FHM1 and supports the view of migraine as a disorder of brain excitatory–inhibitory balance (Pietrobon and Moskowitz, 2013; Vecchia and Pietrobon, 2012).

The lack of effect of FHM1 mutations on GABA release at FS interneuron synapses is puzzling and the underlying mechanism remains unknown. Several possible mechanisms were suggested, including: (i) saturation of the presynaptic calcium sensor; (ii) short duration of the action potential (AP) leading to unaltered AP-evoked presynaptic Ca^{2+} (Ca) influx despite shifted activation of mutant $Ca_V2.1$ channels to lower voltages; and (iii) interneuron-specific $Ca_V2.1$ channels whose gating properties are not affected by the FHM1 mutation (Fioretti et al., 2011; Inchauspe et al., 2010; Tottene et al., 2009; Xue and Rosenmund, 2009). Another interesting question is whether other inhibitory synapses, besides the FS interneuron-pyramidal cell synapse, are unaffected by FHM1 mutations.

Here, we show that the FHM1 R192Q mutation does not affect inhibitory transmission at autapses of cortical FS and other types of multipolar interneurons in microculture, and we investigate the mechanisms underlying this lack of effect despite a dominant role of P/Q channels in controlling GABA release. Our findings support the conclusion that the unaltered inhibitory transmission at multipolar (mainly FS) interneuron autapses is due to the expression of specific $Ca_V2.1$ channels whose gating is barely affected by the FHM1 mutation, and not to near saturation of the presynaptic calcium sensor or short duration of the AP.

Materials and methods

Preparation of cortical neurons in microculture

Cortical neurons were isolated from P0-2 homozygous KI mice carrying the $Ca_V2.1$ FHM1 R192Q mutation (van den Maagdenberg et al., 2004) and wild-type (WT) C57Bl6J mice with the same genetic background following the procedure of Levi et al. (1984). The neurons were cultured on glial microislands (at the density of 6000–25,000 cells/ml) essentially as reported in Brody and Yue (2000) for hippocampal neurons, except for the following details: astrocytes culture medium was basal Eagle's medium (BME) plus 10% fetal bovine serum, 25 mM KCl, 2 mM glutamine and 50 μg/ml gentamicin; neuronal medium was Neurobasal A plus 2% B27 Supplement, 0.5 mM glutamine and 1% PSN Antibiotic mix (all from Gibco); only half volume of astrocytes medium was replaced with neuronal medium to allow the astrocytes to condition the medium before neuron plating. Every 4 days half volume of neuronal medium was changed with a fresh one.

Single cortical neurons grown on glial microislands form synaptic connections onto themselves (autapses). These autaptic connections are by definition monosynaptic, offer an unusually homogeneous population of synapses producing large synaptic responses and solution exchanges can be fast and complete (Bekkers and Stevens, 1991).

All experimental procedures were carried out in accordance with the Italian Animal Welfare Act and with the Use Committee guidelines of the University of Padova and were approved by the local authority veterinary service.

Immunofluorescence assays

Cells grown on glial microislands for 12 days were washed with phosphate buffered saline (PBS), fixed for 20 minutes at room temperature with 4% paraformaldehyde, 4% sucrose in PBS, quenched (0.38% glycine, 0.24% NH_4Cl in PBS, twice for 10 minutes each) and permeabilized with 5% acetic acid in ethanol for 20 minutes at −20 °C. After saturation with 3% bovine serum albumin (BSA) and 10% goat serum in PBS for 20 minutes, samples were incubated with the primary antibodies (diluted in 3% BSA, 10 % goat serum in PBS) for 60 minutes at room temperature. Mouse monoclonal anti-glutamic acid decarboxylase (GAD-67; from Millipore, Billerica, MA, USA) was used at dilution of 1:500; rabbit polyclonal anti-parvalbumin (PV; from Abcam, Cambridge, UK) was used at dilution of 1:300; rat monoclonal anti-somatostatin (SOM; from Millipore) was used at a dilution of 1:100. The specific primary antibodies were detected with Alexa-conjugated secondary antibodies raised in goat (anti-mouse 488, anti-rabbit 647, anti-rat 568; from Life Technologies, Monza, Italy), which were incubated for 60 minutes at room temperature after washing with 3% BSA in PBS; working dilution 1:200 in 3% BSA, 10% goat serum. Coverslips were mounted in mowiol (Sigma). Images were acquired with a DMI6000 inverted epifluorescence microscope (Leica, Germany) equipped with a HCX PL APO 60× oil immersion objective NA 1.4. Differential interference contrast microscopy was used to increase contrast for brightfield imaging of the cultures. Images were acquired with an Orca-Flash4 digital camera (Hamamatsu, Japan).

Electrophysiological recordings and data analysis

Whole-cell patch-clamp recordings were made at room temperature following standard techniques. Electrical signals were recorded through an Axopatch-200B patch-clamp amplifier and digitized using a Digidata 1440A interface and pClamp software (Axon Instruments). Compensation (typically 60–80%) for series resistance was used (5–6 MΩ after compensation).

Microislands containing several glial cells and a single neuron with irregular soma morphology and multiple asymmetrical processes emanating from it (multipolar interneuron; Fig. 1) were selected for recording of evoked postsynaptic currents (PSCs) in voltage-clamp mode (sampling 5 KHz; filter 1 KHz) after 10 to 14 days (DIVs) in culture. Given the well known large diversity of cortical interneurons (Petilla Interneuron Nomenclature Group, 2008)(Rudy et al., 2011), to limit the heterogeneity, cells characterized by fusiform ovoid or spindle-shaped soma with symmetrical processes (Fig. 1) were not considered (to exclude bitufted and bipolar interneurons). Immunofluorescence using antibodies specific for PV and/or SOM revealed that almost all PV-expressing (PV+) interneurons had morphology similar to that of the cells selected for recordings (multipolar interneurons: Fig. 1C) whereas 61% of SOM-expressing (SOM+) interneurons had fusiform ovoid or spindle-shaped soma; the remaining 39% had multipolar morphology similar to that of some of the cells selected for recordings (Fig. 1D).

APs in the unclamped processes were induced by a 2 ms voltage pulse to +20 mV every 10 s from a holding potential of −80 mV. The evoked PSCs were measured at −90 mV.

The pipette solution contained (in mM): 110 K-methanesulfonate, 5 $MgCl_2$, 30 HEPES, 3 EGTA, 4 ATP, 0.5 GTP and 1 cAMP (pH 7.4 with KOH). The extracellular solution contained (in mM): 145 NaCl, 3 KCl, 10 HEPES, 10 glucose, 1 $MgCl_2$, 2 $CaCl_2$ (pH 7.4 with NaOH). In the experiments testing the effect of the peptide toxin ω-AgaIVA (Peptide Institute Inc.), cytochrome C (0.1 mg/ml) was added to the solution.

Although the $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) recorded at −90 mV are inward currents (given the predicted and measured E_{rev} of −69 and −63 mV, respectively), they were easily distinguished from glutamate receptor-mediated excitatory postsynaptic currents on the basis of their slower time course and their complete inhibition by 20 μM bicuculline (Ascent Scientific-Abcam). The currents recorded in the presence of bicuculline were subtracted to all records to obtain the evoked IPSCs (displayed in the figures after blanking 1–3 ms around each stimulus artefact for clarity). After IPSC stabilization (typically 3 minutes after break-in), 5–10 sweeps were averaged to obtain the IPSC amplitude. A liquid junction potential (LJP) of −8 mV should be added to all voltages to obtain the correct membrane potentials (Neher, 1992). Patch-clamp pipettes had resistances of 1.8–2.5 MΩ. The relative number of neurons recorded from WT and KI mice at different DIVs was closely matched.

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