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$Ca_v1.2$ calcium channels modulate the spiking pattern of hippocampal pyramidal cells

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Abstract

$Ca_v1.2$ L-type calcium channels support hippocampal synaptic plasticity, likely by facilitating dendritic Ca^{2+} influx evoked by action potentials (AP) back-propagated from the soma. Ca^{2+} influx into hippocampal neurons during somatic APs is sufficient to activate signalling pathways associated with late phase LTP. Thus, mechanisms controlling AP firing of hippocampal neurons are of major functional relevance. We examined the excitability of CA1 pyramidal cells using somatic current-clamp recordings in brain slices from control type mice and mice with the $Ca_v1.2$ gene inactivated in principal hippocampal neurons. Lack of the $Ca_v1.2$ protein did not affect either affect basic characteristics, such as resting membrane potential and input resistance, or parameters of single action potentials (AP) induced by 5 ms depolarising current pulses. However, CA1 hippocampal neurons from control and mutant mice differed in their patterns of AP firing during 500 ms depolarising current pulses: threshold voltage for repetitive firing was shifted significantly by about 5 mV to more depolarised potentials in the mutant mice ($p<0.01$), and the latency until firing of the first AP was prolonged (73.2±6.6 ms versus 48.1± 7.8 ms in control; $p<0.05$). CA1 pyramidal cells from the mutant mice also showed a lowered initial spiking frequency within an AP train. In control cells, isradipine had matching effects, while BayK 8644 facilitated spiking. Our data demonstrate that $Ca_v1.2$ channels are involved in regulating the intrinsic excitability of CA1 pyramidal neurons. This cellular mechanism may contribute to the known function of $Ca_v1.2$ channels in supporting synaptic plasticity and memory.

Keywords: Neuronal excitability; Action potential; Repetitive firing; CA1 pyramidal cells; $Ca_v1.2$ channel

Introduction

L-type calcium channel-mediated Ca^{2+} influx regulates a variety of functions in the CNS. For example, it contributes to resting intracellular Ca^{2+}, is involved in neuronal plasticity, supports memory, and controls gene expression (Grover and Teyler, 1990; Bito et al., 1996; Impey et al., 1996; Magee et al., 1996; Graef et al., 1999; Morgan and Teyler, 1999; Borroni et al., 2000; Dolmetsch et al., 2001; Woodside et al., 2004). Two L-type isoforms are generally prevalent throughout the CNS: $Ca_v1.2$ and $Ca_v1.3$. In the neocortex and hippocampus, the vast majority belong to the $Ca_v1.2$ subtype (Hell et al., 1993; Sinnegger-Brauns et al., 2004). Evidence showing its dominance on the functional level came recently from two studies using a genetic approach: we have previously shown that mice lacking the $Ca_v1.2$ channel in hippocampal pyramidal cells exhibit defects in spatial learning, late phase LTP (L-LTP), CRE-mediated gene expression and related protein synthesis (Moosmang et al., 2005), while no such phenotypes were observed in mice lacking the $Ca_v1.3$ isoform (Clark et al., 2003). L-type channel activity supporting LTP and, likely, memory formation may arise from invading back-propagated somatic APs (bAP) (Spruston et al., 1995; Hoffman et al., 1997; Markram et al., 1997; Kampa et al., 2006). Moreover, somatic APs alone are (i) sufficient to activate signalling mechanisms supporting the protein synthesis-dependent late phase of LTP (L-LTP), and (ii) capable of converting decremental early phase LTP into stable L-LTP (Dudek and Fields, 2002). Mechanisms involved in the shaping of AP trains are, therefore, of major

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functional relevance. The role of L-type calcium channels in generating repetitive firing of neurons in the CNS is not clear. Studies with pharmacological tools yielded contradictory results (Moyer et al., 1992; Whittington and Little, 1993; Pineda et al., 1998; Chen et al., 2005) and, importantly, do not allow to discriminate between individual members of the L-type channel family. Here, we examined the intrinsic excitability of CA1 pyramidal cells from control mice and mice with the $Ca_v1.2$ gene inactivated in principal hippocampal neurons. Cells from the $Ca_v1.2$ mutants showed (i) elevated threshold and latency for inducing repetitive AP firing, and (ii) a reduced spiking frequency. In our hands, the L-type calcium channel blocker isradipine had the same effects on AP firing in hippocampal pyramidal cells from control mice. Moreover, spiking in these cells was facilitated by the L-type calcium channel activator BayK 8644.

In summary, these findings demonstrate that $Ca_v1.2$ L-type channel isoform modulates the spiking pattern of CA1 pyramidal neurons in a way that may, ultimately, support synaptic plasticity and memory formation.

Materials and methods

We used a mouse line ($Ca_v1.2^{HKO}$) with a conditional inactivation of the *CACNA1C* gene in the hippocampus based on the Cre/loxP system. For details regarding its generation, recombination pattern and genotyping see elsewhere (Schwab et al., 2000; Moosmang et al., 2005; Goebbels et al., 2006). Previous analyses of these mice have revealed that the $Ca_v1.2$ channel protein is still detectable up to day p14 of postnatal development despite the fact that Cre activity in pyramidal neurons of the hippocampus starts from around embryonic day 11.5 (Schwab et al., 2000; Moosmang et al., 2005; Goebbels et al., 2006). Therefore, all animals used in our experiments for comparison of control and $Ca_v1.2^{HKO}$ mice were at least 10 weeks of age. Hippocampal pyramidal cells of these mice lack the $Ca_v1.2$ protein. For experiments with dihydropyridines and with small current ramp, young animals 2–3 weeks of age were used.

Animals were handled in accordance with the Directive 86/609/ECC. All necessary precaution was taken in order to minimise discomfort and pain. Mice were deeply anaesthetised with ether and decapitated. The brain was removed quickly into oxygenated ice-cold artificial cerebrospinal fluid (ACSF, composition in smM: NaCl, 125; $CaCl_2$, 2; $MgCl_2$, 1; KCl, 3; $NaHCO_3$, 25; NaH_2PO_4, 1.25; glucose, 10; pH 7.4 with NaOH). Coronal slices (300 μm thick) including the hippocampus were prepared using a vibratome slicer (Microm GmbH, Walldorf, Germany) and transferred into a storage chamber containing ACSF constantly bubbled with 95% O_2/5% CO_2 where they were allowed to recover for half an hour and stored up to 6 h at room temperature.

For electrophysiological recordings, slices were transferred to a recording chamber mounted on upright microscope BX50WI (Olympus, Hamburg, Germany) perfused with ACSF (24 °C) gassed with 95% O_2/5% CO_2. Recordings were made from CA1 neurons visually identified by infrared DIC-videomicroscopy (Dodt and Zieglgansberger, 1990) using a high performance vidicon camera C2400-07 (Hamamatsu, Herrsching, Germany). Patch pipettes were pulled from borosilicate glass capillaries, and had a resistance of 3–5 MΩ when filled with the internal solution containing (in mM): K-gluconate, 120; KCl, 20; $MgCl_2$, 2; Na_2ATP, 2; Na_2GTP, 0.25; HEPES, 10; and pH 7.3 (with KOH) (Jinno et al., 2003).

To prepare acutely isolated hippocampal neurons, mouse brain was removed and cut into halves in ice-cold ACSF. Both hippocampi were then removed and cut into 300 μm thick slices using a vibratome slicer (Microm GmbH, Walldorf, Germany). Slices were transferred into 5 ml ACSF constantly bubbled with 100% O_2 supplemented with 19 U/ml papain and digested for 90 min at 30 °C. Afterwards slices were washed three times with ACSF and allowed to rest in this solution bubbled with 100% O_2 for 10 min up to 5 h at room temperature. Individual slices were triturated just before patch clamp experiment. Trituration solution contained (in mM): NaCl, 125; KCl, 3; $MgCl_2$, 10; HEPES, 10; Na–HEPES, 10; EGTA, 10; $CaCl_2$, 1; kynurenic acid, 2; pH 7.4 (with NaOH). Following trituration, the solution was exchanged for bath solution and cells were allowed to rest for 15 min before patch clamp measurements were started. Bath solution for measurements of calcium current contained (in mM): NaCl, 105; KCl, 3; TEACl, 25; $MgCl_2$, 0.5; $CaCl_2$, 2; HEPES, 10; D-glucose, 10; pH 7.4 (with NaOH). Patch pipettes were filled with the internal solution containing (in mM): CsCl, 135; $MgCl_2$, 2; TEACl, 20; Na_2ATP, 3; Na_2GTP, 0.4; EGTA, 3; HEPES, 10; pH 7.4 (with CsOH).

Unless mentioned otherwise, all chemical were obtained from Sigma. Stock solutions of BayK 8644 (1 mM; Tocris) and (+/−)isradipine (10 mM, Hofmann-LaRoche) were prepared in ethanol and dissolved in ACSF to the final concentration prior to experiment.

All recordings from CA1 pyramidal cells were made with an HEKA EPC 9 amplifier (HEKA, Lambrecht/Pfalz, Germany). Data were acquired, processed and analysed using the Pulse/Pulsefit (HEKA, Lambrecht/Pfalz, Germany) and Origin 7.5 (OriginLab Co., Northampton, MA, USA) software. All values are given as mean±SEM. Student's *t*-test for independent samples was used for statistical comparison and verified by ANOVA and GraphPad PRISM® software. A confidence interval of ≥95% was considered as significant.

Results

Previously, we have demonstrated that inactivation of the *CACNA1C* gene in hippocampal pyramidal cells virtually abolished dihydropyridine-sensitive calcium currents in these cells (Moosmang et al., 2005). Here, we investigated the effect of this loss in $Ca_v1.2$ channel-mediated currents on electrophysiological characteristics of these cells such as resting potential, input resistance, the shape of single action potential (AP) and the pattern of repetitive spiking.

Resting membrane potential measured as the membrane voltage observed with zero current injected was virtually identical in the two genotypes (−64.0±1.0 mV in 17 cells from 17 slices from 6 control animals versus −65.3±0.8 mV in 16