Neuroscience 231 (2013) 413–419

LOSS OF SURVIVIN IN NEURAL PRECURSOR CELLS RESULTS IN IMPAIRED LONG-TERM POTENTIATION IN THE DENTATE GYRUS AND CA1-REGION

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Abstract—In adult mammals, newborn neural precursor cells (NPCs) derived from either the subventricular zone (SVZ) or the subgranular zone (SGZ) migrate into the olfactory bulb and the dentate gyrus (DG), respectively, where some of them mature into excitatory and inhibitory neurons. There is increasing evidence that this neurogenesis process is important for some types of learning and synaptic plasticity and vice versa. Survivin, a member of the inhibitor-of-apoptosis protein (IAP) family, has been suggested to have a central role in the regulation of neurogenesis. The protein is abundantly expressed in nervous tissue during embryonic development while being restricted postnatally to proliferating and migrating NPCs in SVZ and SGZ. Here we examined adult *Survivin*^{Camcre} mice with a conditional deletion of the *survivin* gene in embryonic neurogenic regions. Although the deletion of survivin had no effect on basic excitability in DG and CA1-region, there was a marked impairment of long-term potentiation (LTP) in these areas. Our data support a function of survivin in hippocampal synaptic plasticity and learning and underline the importance of adult brain neurogenesis for proper operation of the hippocampal tri-synaptic circuit and the physiological functions that depend on it.

Key words: survivin, LTP, dentate gyrus, picrotoxin, neurogenesis.

INTRODUCTION

A variety of physiological, pathological and pharmacological stimuli has been shown to regulate distinct steps of adult neurogenesis including proliferation, fate specification, migration, integration and survival (Song et al., 2002; van Praag et al., 2002; Sahay and Hen, 2007) [see (Ming and Song, 2005; Shors, 2008; Zhao et al., 2008; Pathania et al., 2010) for references]. Within the hippocampus proper, the generation of new neurons in the subgranular zone (SGZ) of the dentate gyrus (DG) is a multistep process during which only a subset of neural precursor cells (NPCs) survives and differentiates, thereby gradually developing functional neuronal properties. SGZ neurogenesis continues lifelong although cell production and survival rate decline with aging (Kuhn et al., 1996; Bizon and Gallagher, 2003; Verret et al., 2007). There is increasing evidence that the integration of newborn neurons and their newly formed synaptic connections into existing neural networks is essential for specific brain functions, such as learning and synaptic plasticity (Gould et al., 1999a; Shors et al., 2001; Drapeau et al., 2003; Nissant et al., 2009). Long-term potentiation (LTP) in DG *in vivo* in turn, has been demonstrated to enhance neurogenesis (proliferation and survival of adult-generated neurons) (Bruel-Jungerman et al., 2006, 2007; Chun et al., 2006).

Survivin, an anti-apoptotic protein and mitotic regulator, is widely expressed in mammals during embryonic development, while much less is expressed in adult tissue (Mahotka et al., 1999; Conway et al., 2000; Caldas et al., 2005; Baratchi et al., 2010; Miranda et al., 2012). The protein has been found in all human cancers of the lung, colon, pancreas, prostate and breast, but it is almost absent in normal tissue (Ambrosini et al., 1997). In the adult human CNS, survivin is expressed in the cerebrum, cerebellum, brainstem as well as spinal cord, retina and neuronal precursor cells (Altura et al., 2003). In the adult mouse

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Abbreviations: ACSF, artificial cerebrospinal fluid; αCaMKII, α-calmodulin-dependent kinase II; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANOVA, analysis of variance; CA1, Cornu Ammonis area 1; Cre, cAMP response element; DG, dentate gyrus; EEG, electroencephalography; fEPSPs, excitatory post-synaptic potentials ; HSP90, heat shock protein 90; IAP, inhibitor-of-apoptosis protein; ISI, inter-stimulus interval; I/O, input/output; LTP, long-term potentiation; NFkappa B, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; NPCs, neural precursor cells; PCR, polimerase chain reaction; RM-ANOVA, repeated measures analysis of variance; RT, room temperature; SEM, standard errors of the mean; SGZ, subgranular zone; SVZ, subventricular zone; TBS, theta-burst stimulation.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.10.049

brain, however, survivin expression is restricted to proliferating and migrating precursor cells in the subventricular zone (SVZ), the rostral migratory stream, and the SGZ of the DG (Coremans et al., 2010). To delineate survivin's function in the brain, several knock-out strains have been generated but a complete knockout of the protein led to embryonic lethality (Uren et al., 2000). In one of the conditional strains, survivin is excised under the control of the nestin promoter at embryonic day 10.5 (Jiang et al., 2005) resulting in massive apoptosis of NPC in the CNS and the death of the neonatal mutants a few minutes after delivery, most likely because of respiratory insufficiency (Jessberger and Kempermann, 2003; Jiang et al., 2005; Trouche et al., 2009). In another conditional strain, the *survivin* gene is prenatally inactivated in the neurogenic regions of the brain (Coremans et al., 2010). In these mice (*Survivin*^{Camcre} mice), we found reduced adult neurogenesis and deficits in hippocampus-dependent learning and memory (Coremans et al., 2010).

Because of the specific expression of survivin in the two neurogenic regions, the hippocampus-dependent learning deficits of *Survivin*^{Camcre} mice, and the outstanding importance of adult neurogenesis for learning and synaptic plasticity (Gould et al., 1999a; Shors et al., 2001; Drapeau et al., 2003; Nissant et al., 2009; Coremans et al., 2010; Miranda et al., 2012), we studied hippocampal synaptic plasticity in these mice. Here, we report that *Survivin*^{Camcre} mice have impaired LTP in the DG and CA1-region indicating a central function of survivin in hippocampal synaptic plasticity that is likely to be mediated by survivin's role in neurogenesis.

EXPERIMENTAL PROCEDURES

Animals

The experiments were performed with conditional survivin knockout mice, which were generated by crossbreeding *Survivin*$^{lox/lox}$ mice (Xing et al., 2004) in which the *survivin* gene is flanked by loxP sites, with CaM-Cre transgenic mice [gift of Dr. G. Schütz, Heidelberg, Germany; (Casanova et al., 2001; Belz et al., 2007)], in which Cre recombinase is selectively expressed in the forebrain region under the control of α-calmodulin-dependent kinase II (αCaMKII) promoter. *Survivin*$^{lox/lox}$ mice as control and *Survivin*^{Camcre} mice as survivin conditional knockouts were maintained on a C57B/6:Swiss:129svj 75:12.5:12.5 background.

Mice were group-housed in standard mouse cages in a room with a 12-h light–dark cycle and *ad libitum* access to food and water. Genotyping of tail DNA was performed by polymerase chain reaction (PCR) as described (Casanova et al., 2001; Xing et al., 2004). All animal experiments were approved by the ethics committee of the University of Leuven.

Electroencephalography recordings

EEG recordings were performed as described (Antonucci et al., 2008, 2009). Under anesthesia (Hypnorm / Hypnovel 0.1 ml/10 g, i.p.), a bipolar electrode (two-twisted enamel-insulated nichrome wires, 120-μm-thick) was implanted into the left hippocampus and a ground electrode was placed over the cerebellum. After animals could recover at least 3 days after surgery, EEG recordings were carried out in freely moving mice during a total of about 10 h / animal in five consecutive days. Seizures were detected with custom software written in LabView (Antonucci et al., 2008, 2009).

Electrophysiological recordings

Extracellular field potentials were recorded in the DG and the CA1-region of hippocampal slices prepared as reported previously (Balschun et al., 1999). After decapitation, the hippocampus was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF in mM: NaCl 124, KCl 4.9, $MgSO_4$ 1.3, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.6, D-glucose 10, saturated with 95% O_2, 5% CO_2, pH 7.4). Slices of 400 μm-thickness were prepared by a custom-made tissue chopper and either directly transferred in the recording chamber where they were kept at 32°C (CA1), or first maintained in a holding chamber at room temperature for 1 h (DG). They were permanently gassed with carbogen under both conditions.

Recordings in DG

For recordings in the DG, slices were transferred from the holding chamber into a submerged-type recording chamber (Campden Instrument, UK) and kept there for at least 90 min at 32°C before glass electrodes (3–5 MΩ) were positioned close to the granule cell layer for recording field excitatory post-synaptic potentials (fEPSPs) and tungsten metal electrodes into the SGZ for stimulation. The initial slope of the fEPSP served as a measure of this potential. Correct placement in the medial perforant pathway was confirmed by the presence of paired-pulse depression at 40-ms inter-stimulus interval (ISI). After input/output (I/O) curves had been constructed, the stimulation strength was set to elicit a fEPSP slope of 40% of the maximum. Short-term plasticity was examined by a paired-pulse protocol where two pulses are applied in rapid succession (IPIs of 20, 50, 100, 200, and 500 ms, respectively) at 120-s intervals. After a stable baseline had been established for at least 30 min, LTP was induced by a theta-burst stimulation (TBS) protocol of 15 trains of eight stimuli delivered at 200 Hz, separated by 200 ms, applied three times at 10-min intervals (Balschun et al., 1999). Because $GABA_A$-mediated inhibition in the DG is very strong, the $GABA_A$-receptor antagonist picrotoxin (10 μM) was added to the ACSF.

Recordings in CA1-region

After they have been placed in a submerged-type chamber, slices were continuously perfused with oxygenated ACSF at a rate of 2.4 ml/min. Recordings were performed as described earlier (Balschun et al., 2010). In brief, a glass electrode (filled with ACSF, 3–5 MΩ) was positioned in the apical dendritic layer to record fEPSPs and a lacquer-coated stainless steel stimulation electrode was placed into the CA1 stratum radiatum about 200 μm apart. To assess the basic properties of synaptic responses, input/output curves were constructed. The stimulation strength was adjusted to evoke a fEPSP-slope of 35% of the maximum and kept constant throughout the experiment. Paired-pulse responses were recorded at interpulse intervals of 10, 20, 50, 100, 200 and 500 ms. Thereafter, the LTP-experiment was started by at least 30 min of baseline recording followed by LTP-induction using a TBS protocol (10 trains of four stimuli delivered at 100 Hz, separated by 200 ms).

Statistical analysis

Data are presented as mean and standard errors of the mean (SEM). To test for group differences between LTP time series, analysis of variance with repeated measures (RM-ANOVA) was used (GraphPad Prism 5.01; GraphPad Soft Inc.).