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Developmental profile and properties of sulforhodamine 101—Labeled glial cells in acute brain slices of rat hippocampus

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Abstract

The reliable identification of astrocytes for physiological measurements was always time-consuming and difficult. Recently, the fluorescent dye sulforhodamine 101 (SR101) was reported to label cortical glial cells *in vivo* [Nimmerjahn A, Kirchhoff F, Kerr JN, Helmchen F. Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. Nat Methods 2004;1:31–7]. We adapted this technique for use in acute rat hippocampal slices at early postnatal stages (P3, 7, 15) and in young adults (P24–27) and describe a procedure for double-labeling of SR101 and ion-selective dyes. Using whole-cell patch-clamp, imaging, and immunohistochemistry, we characterized the properties of SR101-positive versus SR101-negative cells in the *stratum radiatum*. Our data show that SR101, in contrast to Fura-2 or SBFI, only stains a subset of glial cells. Throughout development, SR101-positive and SR101-negative cells differ in their basic membrane properties. Furthermore, SR101-positive cells undergo a developmental switch from variably rectifying to passive between P3 and P15 and lack voltage-gated Na^+ currents. At P15, the majority of SR101-positive cells is positive for GFAP. Thus, our data demonstrate that SR101 selectively labels a subpopulation of glial cells in early juvenile hippocampi that shows the typical developmental changes and characteristics of classical astrocytes. Owing to its reliability and uncomplicated handling, we expect that this technique will be helpful in future investigations studying astrocytes in the developing brain.

Keywords: Astrocyte; Sulforhodamine 101; Hippocampus; Imaging; Electrophysiology; GFAP; SBFI; Fura-2

1. Introduction

During the last decade it has been firmly established that astrocytes are not purely supportive for neuronal function, but also modulate the synaptic communication between neurons (Araque et al., 1999; Fiacco et al., 2007; Haydon, 2001; Haydon and Carmignoto, 2006; Kang et al., 1998; Nedergaard, 1994; Newman and Volterra, 2004; Parri et al., 2001; Parri and Crunelli, 2007; Pascual et al., 2005; Schipke and Kettenmann, 2004; Serrano et al., 2006; Verkhratsky et al., 1998; Volterra and Meldolesi, 2005). Recent studies demonstrated that astroglia also plays a central role in the regulation of blood vessel diameter during neuronal activity (Metea and Newman, 2006; Mulligan and MacVicar, 2004; Takano et al., 2006; Zonta et al., 2003). The analysis of astrocytes in the intact tissue with electrophysiological and high-resolution imaging techniques, however, was always hampered by the problem of a reliable identification of this cell type. The identification of astrocytes based solely on morphological criteria, such as somatic size and cellular architecture, hosts the chance to mistakenly include small-sized neurons (Kimelberg, 2004). Immunohistochemical stainings of markers such as glial fibrillary acidic protein (GFAP) or the Ca^{2+}-binding protein S-100β can only be performed after the experiment, are time-consuming and often do not allow an undeniable identification of the cells analyzed in physiological experiments. To overcome this problem, transgenic mice, in which enhanced green fluorescent protein (EGFP) is expressed under the human GFAP promoter have been raised (Hirrlinger et al., 2006; Nolte et al., 2001; Zhuo et al., 1997). However, because astrocytes show very diverse levels of GFAP-expression

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(Kimelberg, 2004), this approach enables the identification of only a subset of astrocytes.

Many studies reported that glial cells take up the membrane-permeable forms of Ca^{2+} indicator dyes such as Fura-2 or Fluo-4 much better than neurons (Dallwig and Deitmer, 2002; Wang et al., 2006), and astrocytes were thus often identified based on the emission patterns of the indicator dyes used. This approach was extended by Dallwig and Deitmer (2002), who have described that neurons and astrocytes in acute brain slices differ in their response to changes in the external potassium concentration. Still, this approach can only identify about 80% of astrocytes and necessitates performing additional Ca^{2+}-imaging experiments.

Recently, the fluorescent dye sulforhodamine 101 (SR101) was reported as a powerful tool for specific labeling of cortical glial cells in the intact brain of juvenile and adult rodents (Nimmerjahn et al., 2004; Wang et al., 2006). In the present study we adapted this technique for use in an acute tissue slice preparation of the rat hippocampus. Because in the CA1 region of the rodent hippocampus, astrocytes undergo considerable changes in channel complement and passive membrane properties during postnatal development (Bordey and Sontheimer, 1997; Kressin et al., 1995; Zhou et al., 2006), we performed the study at different developmental stages during the first 4 weeks after birth (postnatal days 3, 7, 15, and 24–27). Using whole-cell patch-clamp, imaging techniques, and immunohistochemistry, we show that the percentage of SR101-positive cells in the *stratum radiatum* increases during development. Furthermore, SR101-positive cells lack voltage-gated Na^+ currents and change from variably rectifying to passive cells between P3 and P15. At P15, the majority of SR101-labeled cells is positive for the astrocytic marker GFAP. Thus, our data demonstrate that SR101 selectively labels a subset of glial cells in the hippocampus that shows typical characteristics of classical astrocytes.

2. Methods

2.1. Tissue preparation and labeling with SR101

Experiments were carried out on acute tissue slices (250 μm) of rat hippocampi harvested at postnatal days 3, 7, 15, and 24–27 as described earlier (Meier et al., 2006). In brief, animals were decapitated and the hippocampi were rapidly removed. Slices of P3 and P7 animals were sectioned in ice-cold normal artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 20 glucose, bubbled with 95% O_2 and 5% CO_2; pH 7.4). Following sectioning, slices were kept at 34 °C for 20 min in ACSF that contained 0.5–1 μM sulforhodamine 101 (SR101), followed by a 10 min incubation in normal ACSF at 34 °C. Preparation of slices from animals older than 15 days as well as their incubation with SR101 at high temperature was performed in ACSF with a reduced Ca^{2+} concentration (in mM: 125 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 6 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 20 glucose, bubbled with 95% O_2 and 5% CO_2; pH 7.4). Afterwards, all slices were kept at room temperature until they were used for experiments, which were also performed at room temperature. Unless stated otherwise, all chemicals were purchased from Sigma–Aldrich Co. (Taufkirchen, Germany).

2.2. Determination of the density of SR101-labeled cells

For determination of the amount of SR101-positive cells on the total number of cells exhibiting glial morphology, slices were double-labeled with SR101 and the ester form of sodium-binding benzofuran isophthalate (SBFI), a conventional Na^+-selective fluorescent dye that exhibits similar properties as Fura-2 (Meier et al., 2006). To this end, SBFI-AM (800 μM) was repeatedly (1–5 s duration each) pressure-injected through a fine-tipped glass microelectrode into the *stratum radiatum* (Stosiek et al., 2003). Injection was followed by a 45–60 min wash in normal ACSF at room temperature to allow for diffusion and de-esterification of the dye. Stacks of images (31 optical sections at 1 or 1.5 μm thickness) were then taken at a custom build two-photon laser scanning microscope (excitation wavelength at 850 nm) based on an Olympus FV300 system (Olympus Europe, Hamburg, Germany), coupled to a Mai-Tai Broadband laser (Spectra Physics, Darmstadt, Germany) and equipped with two fluorescence detection channels. Fluorescence emission of SBFI was collected between 400 and 590 nm, emission of SR101 was detected between 610 and 630 nm. Maximum intensity projections and analyses of the staining patterns were performed at montages of image stacks using "ImageJ"-software.

2.3. Electrophysiology and immunohistochemistry

Somatic whole-cell recordings were obtained at an upright microscope (Nikon Eclipse E600FN, 60× water immersion objective, N.A. 1.00, Nikon Europe, Düsseldorf, Germany) using an EPC10 amplifier (HEKA Elektronik, Lambrecht, Germany). "PatchMaster"-software (HEKA Elektronik) was used for data acquisition. Some recordings were carried out at a Zeiss Axioscope (Zeiss, Jena Germany, 40× water immersion objective, N.A. 0.80, Olympus Europe, Hamburg, Germany) using an Axopatch 200A and "PClamp 8.2"-software for data acquisition (Molecular Devices, Sunnyvale, CA). The pipette solution contained (in mM): 120 K-$MeSO_3$ or K-gluconate, 32 KCl, 10 HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), 4 NaCl, 4 Mg-ATP and 0.4 Na_3-GTP, 0.1 Alexafluor 488 (Molecular Probes/Invitrogen, Karlsruhe, Germany), pH 7.30. Cells were generally held at membrane potentials of −85 mV. To separate passive conductances from voltage-gated currents, online leak subtraction (P/4) was performed. Data were processed and analyzed by employing "IGOR Pro"-Software (WaveMetrics, Inc., Lake Oswego, OR).

Following electrophysiological recordings, images of fluorescence emission of SR101-labeled (excitation wavelength: 587 nm, emission detected above 602 nm) and Alexa-filled (excitation wavelength: 488 nm, emission detected between 495 and 575 nm) cells were captured by a CCD camera (Spot RT KE, Diagnostic Instruments, Inc., Sterling Hights, MI) and "Spot"-software attached to the microscope. Slices were immediately fixed over night at 4 °C in paraformaldehyde and immuno-