Loss of the Cisternal Organelle in the Axon Initial Segment of Cortical Neurons in Synaptopodin-Deficient Mice

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ABSTRACT

The axon initial segment of cortical neurons contains the so-called cisternal organelle, an enigmatic formation of stacked endoplasmic reticulum and interdigitating plates of electron-dense material. This organelle shows many structural similarities to the spine apparatus, a cellular organelle found in a subpopulation of dendritic spines. Whereas roles in calcium signaling and protein trafficking have been proposed for the spine apparatus, little is yet known about the physiological function of its putative axonal counterpart. Considering the structural similarity of these two organelles, we hypothesized that synaptopodin, a protein essential for the formation of the dendritic spine apparatus, could also be a component of the cisternal organelle. By using immunofluorescence microscopy, we found that synaptopodin is indeed located within the axon initial segments of principal neurons in the mouse neocortex and hippocampus. Pre-embedding immunogold labeling demonstrated a close association of synaptopodin immunoreactivity with the dense plates of cisternal organelles. In synaptopodin-deficient mice, ultrastructural analysis of identified axon initial segments of CA1 pyramidal cells revealed a lack of cisternal organelles similar to the reported lack of spine apparatuses in these mutants. However, in vitro patch clamp recording of mutant neurons showed that the lack of cisternal organelles did not lead to any changes in basic electrophysiological parameters of action potentials. Taken together, our data demonstrate that synaptopodin is an essential component of the cisternal organelle of axons and of the dendritic spine apparatus, two organelles that are structurally and molecularly related. J. Comp. Neurol. 504:441–449, 2007.

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The axon initial segment (AIS) is a specialized micro-compartment of neurons, extending from the axon hillock to the beginning of the myelin sheath. Ultrastructurally, the AIS is characterized by 1) an electron-dense under-coating of the plasma membrane; 2) fascicles of microtubules; and 3) clusters of ribosomes (Palay et al., 1968) (Palay et al., 1968). The AIS is considered to be the site of action potential generation (Eccles, 1964; Palmer and Stuart, 2006) and has been implicated in the sorting of proteins into the axon (Winckler et al., 1999; Nakada et al., 2003).

In addition to these ultrastructural features, the AIS of cortical principal neurons is also characterized by the presence of one or more cisternal organelles, intriguing stacks of smooth endoplasmic reticulum cisterns interdigitated by electron-dense material (Palay et al., 1968; Peters et al., 1968; Kosaka, 1980; Somogyi et al., 1983). Cisternal organelles are typically located close to the...
After perfusion, brains were removed and postfixed for 24 hours in 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Serial frontal sections (50 μm) of the dorsal hippocampus were cut with a Vibratome (VT 1000S, Leica, Bensheim, Germany). Sections were cut in the frontal plane, approximately perpendicular to the longitudinal axis of the hippocampus.

For electrophysiological experiments, synaptopodin-deficient mice (male, 4–8 weeks old; n = 3) and wildtype littersmates (male, 4–8 weeks old; n = 3) were used. All experiments were performed in agreement with the German law on the use of laboratory animals.

**Immunofluorescence labeling**

Immunolabeling was performed as described (Bas Orth et al., 2005). Briefly, free-floating sections were blocked in Tris-buffered saline (TBS; pH 7.5) containing 5% normal goat serum and 0.5% Triton X-100 and subsequently incubated overnight at 4°C in a mixture of a polyclonal rabbit anti-synaptopodin antibody (SE-19, Sigma-Aldrich, St. Louis, MO, cat. no. S9442; 1:1,000) and a chicken anti-βIV-spectrin antibody (1:1,000; gift from M. Komada, Tokyo Institute of Technology, Japan). After washing, sections were incubated with Alexa 488-labeled goat anti-rabbit and Alexa 568-labeled goat anti-chicken secondary antibodies (Molecular Probes, Eugene, OR; 1:1,000). After mounting with anti-fading medium (DAKO® Fluorescent Mounting Medium; Dako, Hamburg, Germany), sections were viewed with a Zeiss LSM510 confocal microscope by using 20× (NA 0.75) and 63× (NA 1.4) objective lenses.

Synaptopodin-positive puncta in AIS were quantified in area CA1. For quantification single optical sections were used. Puncta were segmented by using ImageJ software as previously described (Bas Orth et al., 2005), and the number of puncta was determined in randomly selected βIV-spectrin-positive AIS (250 AIS from n = 5 animals). To correct for differences in AIS-profile length, values were expressed as mean number per 10 μm ± SD.

**Electron microscopy**

For electron microscopy of unlabeled mouse hippocampus, sections postfixed overnight were washed in phosphate buffer (PB), osmicated (0.5% OsO₄ in PB, 30 minutes), dehydrated (70% ethanol containing 1% uranyl acetate), and embedded between liquid release-coated slides and coverslips. Sections containing the CA1 region of the hippocampus were re-embedded in blocks, and serial ultrathin sections were collected on single-slot Formvar-coated copper grids. Ultrathin sections were examined in a Philips electron microscope.

Pre-embedding DAB and immunogold labeling were performed as described (Deller et al., 2002). In brief, after a blocking step, sections were incubated with anti-phosphorylated-IκBα antisera (Ser32, Cell Signaling Technology, Frankfurt, Germany; 1:1,000) or anti-synaptopodin antisera (NT-61, Mundel et al., 1997; 1:2,000) in Tris buffer (TB) containing 1% bovine serum albumin. For the detection of the anti- phospho-IκBα antibody, a secondary biotinylated antibody was used (1:250 biotinylated anti-rabbit; 2 hours; room temperature; Vector, Burlingame, CA). After rinsing in TB, sections were incubated in avidin-biotin-peroxidase complex (ABC-Elite, Vector) for 2 hours at room temperature. Following three subsequent washes, the sections were reacted for 5–10 minutes in a nickel/diaminobenzidine solution (0.05% 3,3’-diaminobenzidine, 0.02% nickel-ammonium}

**MATERIALS AND METHODS**

**Animals and tissue preparation**

For the morphological part of the study, adult male C57BL/6J mice (3 months; n = 12), synaptopodin-deficient mice (Deller et al., 2003; 3 months; n = 7), and adult male Thy1-GFP transgenic mice (Feng et al., 2000; 3 months; n = 3), housed under standard laboratory conditions, were used. Mice were deeply anesthetized with an overdose of pentobarbital (300 mg/kg body weight) and transcardially perfused with 0.9% NaCl followed by fixative: 1) for immunofluorescence, the fixative contained 4% paraformaldehyde; 2) for electron microscopy of unlabeled sections, the fixative consisted of 1% paraformaldehyde, 2.5% glutaraldehyde, 0.1% picric acid; 3) for electron microscopy of synaptopodin-immunostained sections (diaminobenzidine [DAB]-labeled sections) a fixative containing 4% paraformaldehyde and 0.5% glutaraldehyde was chosen; and 4) for electron microscopy of synaptopodin-stained sections (immunogold labeling), a fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 15% picric acid in 0.1 M phosphate-buffered saline (PBS; pH 7.4) was used. After perfusion, brains were removed and postfixed for 24
SYNAPTOPODIN^-/- MICE LACK CISTERNAL ORGANELLES

Characterization of antibodies

The affinity-purified polyclonal SE-19 antibody was obtained by immunization of rabbits with keyhole limpet hemocyanin (KHL)-conjugated synthetic peptides corresponding to amino acids 184–202 of rat brain synaptopodin (manufacturer’s technical information). By Western blot analysis, this antibody detects the 110-kDa renal Synpo-long isoform in cytosolic fractions from isolated glomeruli and the 100-kDa neuronal Synpo-short isoform in the brains of adult rats and mice (Mundel et al., 1997). No immunostaining was seen in sections from synaptopodin knockout mice (Deller et al., 2003).

The previously described polyclonal anti-βIV-spectrin antibody (Komada and Soriano, 2002) was raised against the variable region of βIV-spectrin (amino acids 2,171–2,345 of βIVΣ1-spectrin) and absorbed against βIVspectrin-null brain. In Western blots of wildtype brain, the antibody detects a major band at 160 kDa. No band is detected in Western blots of βIVspectrin-null brain.

The phospho-epitope-specific polyclonal anti-pIκBα antibody was produced by immunization of rabbits with a keyhole limpet hemocyanin (KLH)-conjugated synthetic phosphopeptide corresponding to residues arround Ser32 of human IκBα (single letter code LDRHDsGLDSMKDC, in which the lowercase “s” is phosphoserine 32; Cell Signaling Technology, personal communication). Specificity was previously tested by preabsorption with synthetic peptide and by dephosphorylation of the endogenous antigen (Schultz et al., 2006).

Whole-cell patch-clamp recording

All experiments were performed with the experimenter blind to the genotype of the animals. Mice were killed by decapitation, and transverse hippocampal slices (300 μm) were cut on a Vibratome (Leica) in ice-cold physiological saline containing (in mM): 87 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 10 glucose, 75 sucrose, 0.5 CaCl2, and 7 MgCl2. Slices were kept in the sucrose-containing medium at 35°C for 30 minutes after cutting and at room temperature during subsequent storage. All solutions were continuously bubbled with 95% CO2/5% O2 to maintain a pH of 7.35. For recording, slices were transferred to a recording chamber and superfused with physiological saline at 32°C containing (in mM): 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 10 glucose, 2 CaCl2, and 1 MgCl2.

Patch pipettes (5–7 MΩhm) were pulled from thick-walled borosilicate glass tubing and filled with a solution containing (in mM): 140 K-glucosate, 5 NaCl, 0.5 EGTA, 10 4-(2-hydroxyethyl)piperazine-1-ethansulfonic acid (HEPES), 0.4 Na-GTP, and 2 Mg-ATP (pH 7.3). Individual neurons were visualized by differential interference contrast infrared videomicroscopy (Döld and Ziegleransberger, 1990) by using a CCD camera (PCO CCD Imaging, Kelheim, Germany) mounted on an upright microscope (Axioskop FS; Zeiss, Oberkochen, Germany). CA1 pyramidal cells were approached under visual control by maintaining a moderate positive pressure in the patch pipette. Membrane currents were recorded in the whole-cell configuration of the patch-clamp technique by using an EPC-10 amplifier (Heka, Lambrecht, Germany). Patch pipette capacitance and cell capacitance were canceled, and series resistance was compensated by at least 80% by using the internal compensation circuits of the amplifier. The sampling frequency was 10 kHz. Currents were filtered at 5 kHz by using the internal low-pass filter of the amplifier. Data were digitized and stored online by using the Pulse software (Heka) and analyzed offline by using Mini-Analysis (Synaptosoft, Decatur, GA). Statistical significance was tested by using the nonparametric Mann-Whitney U test (SPSS for MS Windows, SPSS, Chicago, IL). Tests were two-tailed, and significance was set at P < 0.05.

Digital illustrations

Confocal images were exported from the Zeiss LSM image browser and stored as TIFF files. For overview figures (Fig. 2A,B), confocal image stacks were projected onto the xy-plane by using the projection function of the Zeiss LSM image browser. Electron micrographs were digitized by using a scanner (DuoScan T2500, Agfa, Cologne, Germany) and stored as TIFF files. Figures were prepared by using Photoshop 6.0 graphics software (Adobe, San Jose, CA). Image brightness, contrast, and sharpness were adjusted.

RESULTS

Synaptopodin is present in the axon initial segments of cortical neurons

Immunofluorescence labeling for synaptopodin and the membrane adaptor protein βIV-spectrin, a marker for the AIS (Bergha et al., 2000; Bennett and Baines, 2001), were combined. In the hippocampus, confocal analysis of double-labeled brain sections revealed one or more synaptopodin-positive puncta within βIV-spectrin-positive profiles in the fascia dentata and in hippocampal subfields CA3 and CA1 (Fig. 1A–C). Synaptopodin-positive puncta located within βIV-spectrin-labeled AIS were significantly larger and more elongated than synaptopodin-puncta observed in dendritic layers (data not shown). A quantitative analysis in area CA1 revealed 3.0 ± 0.9 (mean ± 5D) synaptopodin-puncta per 10 μm of AIS. In the principal cell layers of the hippocampus, the vast majority of synaptopodin-puncta was localized to βIV-spectrin-positive AIS, indicating that dendritic synaptopodin contributes little to the immunoreactivity observed in these layers.

In the neocortex (Fig. 1D), a similar co-localization of synaptopodin and βIV-spectrin-positive AIS profiles was found: one or more synaptopodin-puncta were detected in
βIV-spectrin-positive AIS in all layers and all cortical regions investigated, including portions of the frontal, temporal, and parietal cortex. Because the AIS of neurons located in superficial cortical layers intermingle with dendritic processes of neurons located in deeper cortical layers, synaptopodin-puncta located in dendrites and synaptopodin-puncta located in AIS were regularly observed in the same cortical layers (Fig. 1D). Similar to the situation in the hippocampus, synaptopodin-positive structures located within AIS were larger and more elongated than synaptopodin-puncta observed in the adjacent neuropil, presumably within dendritic spines. Especially in cortical layer V, synaptopodin-labeled structures had an elongated rod-like shape that extended over several micrometers in individual cases.

To analyze whether synaptopodin is restricted to the AIS or whether it can also be found in more distal portions of the axon, brain sections from Thy1-GFP transgenic mice (Feng et al., 2000; Bas Orth et al., 2005) were employed and stained for βIV-spectrin as well as for synaptopodin. In these sections, individual hippocampal principal neurons and their axons are enhanced green fluorescent protein (GFP) positive, which makes it possible to localize synaptopodin in different portions of the axon. Again, the AIS was positive for βIV-spectrin and contained several synaptopodin-immunoreactive puncta (Fig. 2). In contrast, βIV-spectrin-negative portions of the axon, i.e., more distal portions of the axon, were devoid of synaptopodin (Fig. 2). We conclude that axonal synaptopodin is restricted to the AIS.

**Synaptopodin is associated with the cisternal organelle of CA1 pyramidal cells**

After demonstrating synaptopodin in the AIS at the light microscopic level, we used electron microscopy to investigate whether synaptopodin is associated with the cisternal organelle. This organelle typically consists of several cisterns of endoplasmic reticulum interdigitated by plates of electron-dense material (Palay et al., 1968).

First, unlabeled hippocampal sections were investigated to identify the cisternal organelle in the AIS of mouse CA1 pyramidal cells. Similar to the situation in the rat cortex (Palay et al., 1968), a typical cisternal organelle found in the AIS of these cells consisted of several dense plates and cisterns of endoplasmic reticulum. In the case of the CA1 pyramidal cell illustrated in Figure 3, the axon could be followed for approximately 10 μm from its origin at

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**Fig. 1.** Synaptopodin is present in axon initial segments of cortical neurons. A–D: Double-immunofluorescence labeling for synaptopodin (green) and βIV-spectrin (magenta) reveals synaptopodin-positive puncta (arrows) within each βIV-spectrin-positive axon initial segment in the fascia dentata (A), hippocampal area CA3 (B) and CA1 (C), and neocortex (D). Images represent single confocal sections. GCL, granule cell layer; H, hilus; l, stratum lucidum; ML, molecular layer; o, stratum oriens; p, stratum pyramidale; rad, stratum radiatum. Scale bar = 10 μm in A–D.
the axon hillock. The AIS could be readily identified on the basis of its electron-dense undercoating and the characteristic bundling of microtubules (Fig. 3A). Within the AIS a cisternal organelle was found consisting of four dense plates and three cisterns of endoplasmic reticulum (Fig. 3B).

Next, synaptopodin-immunostained hippocampal sections were analyzed to localize synaptopodin in AIS. Pre-embedding immunogold labeling was employed for subcellular localization. In this material, synaptopodin immunoreactivity in the AIS was tightly associated with cisternal organelles (Fig. 4A) and predominantly found over the dense plates between the cisterns of endoplasmic reticulum. This ultrastructural localization very much resembled the situation in dendritic spines, where synaptopodin is associated with the dense plates of the spine apparatus (Deller et al., 2000a, 2003).

Synaptopodin-deficient mice lack cisternal organelles

The tight association of synaptopodin with the cisternal organelle raised the question of whether synaptopodin is essential for its formation, as has been demonstrated for the spine apparatus (Deller et al., 2003). To address this issue, AIS of wildtype and synaptopodin-deficient mice were analyzed, and the relative frequency of cisternal organelles within AIS profiles was quantified. To verify the detection of AIS in ultrathin sections, we employed an antiserum against phosphorylated 14-3-3 (Ser32), a robust marker for the AIS that can be used for electron microscopic studies (Schultz et al., 2006; Fig. 4B).

The DAB immunoprecipitate labeled the cytoplasm within the AIS in wildtype as well as synaptopodin-deficient mice (Fig. 4C,D). Of note, cisternal organelles...
were not obscured by the DAB immunoprecipitate and could readily be detected in wildtype specimens (Fig. 4C). In hippocampal area CA1 of wildtype mice, 4.6 ± 1.0 (mean ± SEM) cisternal organelles were found per 100 AIS profiles. Although single cisterns of smooth endoplasmic reticulum were occasionally observed in the AIS of synaptopodin-deficient mice, not a single cisternal organelle was detected.

**The generation of action potentials is not altered by the absence of cisternal organelles**

One of the physiological roles of the AIS is the initiation of action potentials (Eccles, 1964; Palmer and Stuart, 2006). To test whether the lack of cisternal organelles influences the generation of action potentials, we performed whole-cell patch-clamp recordings of synaptopodin-deficient and wildtype hippocampal CA1 pyramidal cells. Recordings from 43 cells (−/−: n = 20, +/+: n = 23) having a resting membrane potential below −60 mV and a mean (± SD) input resistance of 518 ± 139 MOhm were analyzed. Analysis included spike initiation threshold and spike form at slightly superthreshold depolarizing pulses (Fig. 5A), as well as spike adaptation during supermaximal depolarizing pulses (Fig. 5B). All cells were clamped to −80 mV before the application of depolarizing current injections.

All cells studied revealed clear adaptation of spike frequencies during prolonged supermaximal depolarization
**DISCUSSION**

The cisternal organelle is a characteristic feature of the AIS of cortical principal neurons. Although it was described almost 40 years ago (Palay et al., 1968; Peters et al., 1968), its molecular composition and physiological function are poorly understood. Our present studies on the cisternal organelle can be summarized as follows: 1) the actin-associated molecule synaptopodin is a molecular marker of the cisternal organelle; 2) synaptopodin-positive cisternal organelles are regular components of the AIS of cortical principal cells; 3) synaptopodin-deficient mice lack cisternal organelles in the AIS; and 4) lack of the cisternal organelle does not lead to significant changes in action potential generation. We conclude from these findings that synaptopodin is essential for the formation of a cisternal organelle. Because synaptopodin is also required for the formation of a dendritic spine apparatus, our findings strengthen the concept that these two organelles are structurally and molecularly related.

**Synaptopodin is a molecular component of the cisternal organelle**

In previous studies on the distribution of synaptopodin in the hippocampus, a string-like labeling of synaptopodin-positive puncta was reported in hippocampal principal cell layers (Deller et al., 2000a). Although originally believed to represent synaptopodin-positive spine apparatuses in dendritic spines, an axonal localization of synaptopodin was also discussed on the basis of the morphological similarity between the spine apparatus and the cisternal organelle (Deller et al., 2000a). In the present study, we could verify such an axonal localization of synaptopodin, by using double-immunofluorescence labeling of synaptopodin and βIV-spectrin, as well as synaptopodin staining of Thy1-GFP mouse hippocampus (Feng et al., 2000; Bas Orth et al., 2005). By using pre-embedding immunogold labeling, an association of the axonal synaptopodin-positive puncta with the cisternal organelle could also be shown. Thus, synaptopodin is a molecular component of the cisternal organelle and can be employed to label this organelle in the AIS of neurons.

**The cisternal organelle is a regular feature of the axon initial segment of telencephalic principal neurons**

The association of synaptopodin with the cisternal organelle makes it possible to estimate the number of synaptopodin-positive cisternal organelles in AIS of hippocampal and neocortical principal cells by using light microscopy. In our material almost every AIS contained at least one synaptopodin-positive structure, indicating that synaptopodin-positive cisternal organelles are a regular feature of the AIS of telencephalic principal cells. Based on our light microscopic observations, these neurons have on average 3.0 cisternal organelles per 10 µm AIS. Thus, this organelle appears to be a very regular and common feature of telencephalic principal axons, occurring more frequently than previously estimated on the basis of conventional electron microscopic analysis (Peters et al., 1968).

**Synaptopodin is an essential component of the cisternal organelle**

The tight association between synaptopodin and the cisternal organelle raises the question of whether synaptopodin is required for its formation. This question was addressed by analyzing the AIS of synaptopodin-deficient mice (Deller et al., 2003). This approach is not trivial, because AIS are rarely encountered in unstained brain sections, and the frequency with which cisternal organelles are observed within axonal profiles is very low (Peters et al., 1968). Thus, we focused our investigations on the AIS of hippocampal CA1 pyramidal cells. These cells are densely packed in a single cell layer and have parallel axons, which are oriented toward the alveus. In addition, we identified AIS by using an antibody against

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### Table 1: Comparison of Synaptic Properties Between Wildtype and Synaptopodin-Deficient Mice

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<thead>
<tr>
<th>Property</th>
<th>Wildtype (mean ± s.d.)</th>
<th>Synaptopodin -/- (mean ± s.d.)</th>
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<tbody>
<tr>
<td>AP amplitude [mV]</td>
<td>96.2 ± 11.7</td>
<td>101.4 ± 12.2&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>AP threshold [mV]</td>
<td>-45.6 ± 6.7</td>
<td>-47.9 ± 7.7&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>AP half-width [ms]</td>
<td>0.87 ± 0.10</td>
<td>0.84 ± 0.11&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>AHP amplitude [mV]</td>
<td>-5.1 ± 4.8</td>
<td>-7.1 ± 2.1&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>APs per 1s depolarization</td>
<td>30.0 ± 10.6</td>
<td>36.0 ± 8.5&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maximum firing rate [Hz]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>125.0 ± 60.2</td>
<td>122.3 ± 63.8&lt;sup&gt;n.s.&lt;/sup&gt;</td>
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<sup>n.s.</sup> not significant. Scale bar = 100 ms, 20 mV in A,B.

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(Fig. 5B). Cells from synaptopodin-deficient mice and those from wildtype littermates revealed no significant differences in spike initiation threshold, action potential form, i.e., peak amplitude, width at 30% amplitude, or peak amplitude (Fig. 5C) and integral of the afterhyperpolarization (AHP) following repolarization. Mean number of APs elicited during 1 second supermaximal depolarization and the maximum firing rate as determined from the temporal separation of the first two APs of the trains also revealed no apparent difference between the two groups. * determined from first two APs. n.s., not significant.
have been shown to determine the afterhyperpolarization action potential. Calcium-dependent ionic conductances, gold labeling and enzyme histochemistry (Benedeczky et al., 1994), but not in cerebellar Purkinje cells (Somogyi and Hamori, 1976), motoneurons of the spinal cord (Conradi, 1969), neurons of the medial accessory olive (de Zeeuw et al., 1990), or the trigeminal nucleus (Westrum, 1993). This distribution pattern of the cisternal organelle corresponds nicely to the expression pattern of synaptopodin, which is expressed exclusively by neocortical and hippocampal neurons but not in the cerebellum or brainstem (Mundel et al., 1997; Deller et al., 2002). Thus, neurons that do not express synaptopodin, either naturally or because of gene targeting, do not contain cisternal organelles.

The cisternal organelle of the axon initial segment and the spine apparatus of dendritic spines share morphological and molecular features

It has been pointed out on the basis of electron microscopic data that the cisternal organelle and the spine apparatus are morphologically very similar (Peters et al., 1968; Kosaka, 1980; Benedeczky et al., 1994): Both organelles contain stacks of smooth endoplasmic reticulum that are interconnected by dense plates (Gray, 1959a,b; Palay et al., 1968; Peters et al., 1968; Spacek, 1985). In addition, both the spine apparatus (Fitiqova et al., 1983; Sharp et al., 1993; Korkotian and Segal, 1998) and the cisternal organelle (Benedeczky et al., 1994) have been implicated in local calcium trafficking. Our finding that synaptopodin is an essential component of both the cisternal organelle and the spine apparatus strengthens the hypothesis that these two organelles share many of their basic features. These morphological and functional similarities, however, do not preclude the involvement of the two organelles in very different and site-specific biological processes that are determined by the local molecular microenvironment of the spine and the AIS, respectively.

What could be the function of the cisternal organelle?

The available data suggest that the cisternal organelle is a Ca\textsuperscript{2+}-storing compartment, because it contains SERCA-type Ca\textsuperscript{2+} pumps as demonstrated by immunogold labeling and enzyme histochemistry (Benedeczky et al., 1994). Based on its location in the AIS, the site of action potential generation, we speculated that it could be important for some of the physiological parameters of the action potential. Calcium-dependent ionic conductances have been shown to determine the afterhyperpolarization following action potentials and spike accommodation over prolonged depolarizations (Madison and Nicol, 1984; Powers et al., 1999). We therefore analyzed action potentials of synaptopodin-deficient pyramidal cells in hippocampal area CA1 by using whole-cell patch-clamp recordings and compared these data with the electrophysiological properties of littermate controls. Because we did not observe significant differences between the two groups, we conclude that the cisternal organelle does not play a critical role in action potential generation.

An alternative hypothesis suggests that the cisternal organelle might be involved in GABAergic transmission at the AIS (Benedeczky et al., 1994). This hypothesis is based on the proximity of GABAergic synaptic terminals and cisternal organelles at AIS (Sloper, 1973; Benedeczky et al., 1994). In this context it is interesting that telencephalic neurons containing a cisternal organelle receive numerous axoaxonal, GABAergic synaptic inputs, whereas AIS of cerebellar Purkinje cells and spinal motoneurons that do not have this organelle receive only a few GABAergic synapses (Palay and Chan-Palay, 1974; Kosaka, 1980; Howard et al., 2005). Thus, the number of GABAergic terminals on AIS correlates with the distribution of the cisternal organelle and synaptopodin. Whether the cisternal organelle is, in fact, involved in the modulation of GABAergic transmission at the AIS can now be tested by using synaptopodin-deficient mice.

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