Sortilin-related Receptor with A-type Repeats (SORLA) Affects the Amyloid Precursor Protein-dependent Stimulation of ERK Signaling and Adult Neurogenesis

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Sortilin-related receptor with A-type repeats (SORLA) is a sorting receptor that impairs processing of amyloid precursor protein (APP) to soluble (s) APP and to the amyloid β-peptide in cultured neurons and is poorly expressed in patients with Alzheimer disease (AD). Here, we evaluated the consequences of Sorla gene defects on brain anatomy and function using mouse models of receptor deficiency. In line with a protective role for SORLA in APP metabolism, lack of the receptor resulted in increased amyloidogenic processing of endogenous APP and in aggravated plaque deposition when introduced into PDAPP mice expressing mutant human APP. Surprisingly, increased levels of sAPP caused by receptor deficiency correlate with profound stimulation of neuronal ERK signaling and with enhanced neurogenesis, providing in vivo support for neurotrophic functions of sAPP. Our data document a role for SORLA not only in control of plaque burden but also in APP-dependent neuronal signaling and suggest a molecular explanation for increased neurogenesis observed in some AD patients.

Sortilin-related receptor with A-type repeats (SORLA), also known as LR11 or SORL1, is a sorting receptor that controls intracellular transport and processing of the amyloid precursor protein (APP) in cultured neurons (1–4). The receptor shuttles between Golgi, plasma membrane, and endosomes (5), and determines residence time of the precursor protein in the various intracellular compartments (3). Most importantly, the receptor promotes retention of APP in subcellular compartments less favorable for processing and thereby reduces the extent of proteolytic breakdown into both amyloidogenic and non-amyloidogenic products. Consistent with its protective role in APP catabolism, increasing SORLA expression in cells reduces conversion of APP to the amyloid β-peptide (Aβ) and soluble (s) APP fragments, while low levels of receptor activity accelerate generation of these processing products (3, 4, 6).

Recently, a possible role for SORLA as a risk factor for sporadic Alzheimer disease (AD) was supported by the association of inherited gene variants with the occurrence of this disease in several populations (7, 8). These findings support earlier studies that reported low levels of Sorla gene expression in patients suffering from sporadic AD, but not in individuals with familial forms of the disease that are caused by defects in genes encoding APP or presenilin 1 and 2 (9, 10).

A substantial amount of data correlate SORLA activity with APP processing and Aβ production rates in cell cultures. Still, the normal physiological role of SORLA-dependent regulation of APP processing in vivo and the pathophysiological consequences of insufficient receptor activity in the brain remain poorly understood.

Here, we used alternative mouse models with targeted Sorla gene disruption to address the molecular and pathophysiological consequences of impaired SORLA activity for neuronal function and AD pathology in vivo. Our findings identified a distinct increase in Aβ production and amyloid plaque formation in transgenic mouse models of AD lacking receptor expression. In addition, we uncovered stimulation of mitogen-activated protein (MAP) kinase signaling pathways and enhanced neurogenesis, possibly caused by high levels of soluble APP fragments found in the SORLA-deficient brain. These findings highlight an important role for SORLA in the regulation of physiological APP-dependent signaling pathways; neuronal processes that are likely to be altered in AD patients suffering from insufficient receptor activity.

**EXPERIMENTAL PROCEDURES**

Materials—Antibodies directed against SORLA were produced in house (1227), provided by collaborators (MX-02, Gerd Multhaup) (11), or obtained from com-
crometer suppliers (4G8, Signet; 22C11, Chemicon; JP18957, IBL Hamburg). Antisera directed against activated caspase 3, AKT/pAKT, and ERK/pERK (Cell Signaling Technology), as well as soluble APPs (Sigma-Adrich, cat. no. S9564) and Aβ(1–40) (Bachem, cat. no. H-1194) were commercially available.

**APP Processing Products**—The amounts of APP, soluble APP, and Aβ fragments in hippocampal lysates or extracts from primary neurons were determined by standard Western blotting and following protein separation by 6% SDS-PAGE. The blots were developed with SuperSignal West Femto Chemiluminescent Substrate (Pierce) detected with the LAS1000 imaging system (FUJI Photo Film Co., Japan). Band intensities were quantified using Advanced Image Data Analyzer (AIDA) software (Rays-test, Straubenhardt, Germany) version 3.51. The relative units represent LAU (local area units) per millimeter² minus background. Human Aβ40 was quantified using a commercial ELISA kit (KHB3482, BIOSOURCE). Unless stated otherwise, the statistical significance of data was determined using the Student’s t test.

**Determination of Plaque Load**—Three to six hippocampal sections from each (PDAPP, Sorla+/-) (10 mo, n = 13) and (PDAPP, Sorla-/-) (10 mo, n = 9) mouse were randomly chosen and stained with antibodies directed against Aβ (MX-02, 4G8; 1:500 dilution). The quantification of relative density of immunoreactivity was performed using the software program Cell F (Olympus) in single optical images and all data related to the mean value of the (PDAPP, Sorla+/-) controls in the respective data set. To do so, ×4-magnification images of the sections were taken. After defining a constant color threshold, hippocampus, and subiculum on the sections were manually delineated as region of interest, images were binarized, and relative staining intensity of the surface area in the defined region of interest calculated. Levels of statistical significance were calculated by the Mann-Whitney U test. All data are given as means ± S.E.

**Animal Experimentation**—The generation of SORLA-deficient mice by targeted gene disruption has been described before (3). This line was kept on a hybrid (129SvEmcTer × C57BL/6N) genetic background. A second receptor null line on a (129/Ola × C57BL/6N) background was generated independently through insertional mutagenesis of the murine Sorla gene locus by a lacZ reporter gene (12) by William C. Skarnes (Welch Trust Sanger Center, Cambridge, UK). Both lines gave identical results. All studies involving animals were performed in accordance with institutional guidelines. For electrophysiology, horizontal hippocampal brain slices (300-μm thick) were prepared according to standard procedures and field potential recordings performed with low resistance patch-clamp electrodes (13). Field EPSPs were recorded in stratum radiatum in area CA1. Schaffer collaterals were stimulated with a frequency of 0.1–0.05 Hz. Stimulation strength was adjusted to 30–50% of the strength needed to evoke population spikes. LTP was induced by four tetani of high frequency stimulation at 100 Hz for 1 s with 20 s intertrain intervals. The magnitude of LTP was determined by averaging the responses collected during the last 5 min of each experiment. Data are expressed as means ± S.E.

**Immunohistology**—Brains were fixed in situ in 4% formalin by transcardiac perfusion, post-fixed at 4°C for 24 h, and stored in 30% sucrose until further processing. For each animal, one hemibrain was cut into 40-μm thick free-floating sagittal sections, while the second hemibrain was embedded in paraffin and cut into 5-μm sections. The staining of paraffin sections with thionine (Nissl staining) or immunohistological detection of acetylcholine transferase (1:200; Chemicon, Schwalbach, Germany), macrophage/microglial marker F4/80 (1:100; Serotec, Düsseldorf, Germany), astrocytic marker GFAP (glial fibrillary acidic protein) (1:1000 Advanced Immunochemical Inc., Long Beach, CA) on free-floating sections was performed according to standard protocols using biotinylated secondary antibody (1:200; Vector Laboratories, Burlingame, CA) and the ABC Elite kit (Vector Laboratories) for visualization. For fluorescence microscopy (GFAP), sections were incubated with a secondary antibody conjugated with Alexa 488 (1:250; Molecular Probes, Karlsruhe, Germany) for 4 h, mounted with Vectashield (Vector Laboratories), and viewed with a laser scanning confocal microscope. DNA fragmentation in apoptotic nuclei was detected by labeling with the in situ cell death detection kit (Roche Applied Sciences GmbH, Mannheim, Germany) according to the manufacturer’s recommendations. For immuno-electronmicroscopy detection of APP, dissected hippocampi of one wild-type and one knockout mouse were fixed in 4% formaldehyde, 0.5% glutaraldehyde in 0.1M phosphate buffer, embedded in 10% gelatin, and infiltrated with 2.3 M sucrose. Frozen samples were trimmed to the region of the neuronal cell layers, and ultrathin cryosections were obtained according to Tokuyasu. APP was detected by polyclonal anti-APP antibody 1227 (diluted 1:250). For signal detection, 12 nm colloidal gold-AffiniPure goat anti-rabbit IgG, EM grade (Jackson Immuno Research Lab., Inc.) was used. Labeled Golgi fields were examined on a Zeiss 910 electron microscope equipped with an 1k × 1k CCD camera (Proscan) and analyzed with the analySIS 3.2 software (Soft Imaging System, Münster, Germany) for counting grains of gold and Golgi area determination.

**Quantification of Neurogenesis**—The proliferation and survival of newborn neurons were determined by BrdUrd incorporation analysis following a 24 h or 4-week chase period, respectively. Animals received a single (proliferation) or 3-day repeated (survival) intraperitoneal dose of 50 μg of BrdUrd (Sigma)/g body weight at 10 mg/ml in 0.9% NaCl. Brains were fixed in 4% PFA, infiltrated in 30% sucrose, and sectioned at 40 μm. The number of BrdUrd-labeled cells was determined by immunohistology using rat anti-BrdUrd (1:500; Harlan Seralab, Indianapolis, IN), followed by biotinylated donkey anti-rat (1:500; Dianova) and the peroxidase detection system (ABC Elite kit) with nickel-intensified diaminobenzidine as chromogen. The sections were counted in a one-in-six series through a ×40 objective, and multiplied by 6 to give an estimate for the total number of BrdUrd-positive cells per entire dentate gyrus.

**RESULTS**

Previously, we had reported the generation of a mouse model with targeted Sorla gene disruption. Loss of receptor activity was shown to increase the overall production of sAPP and Aβ, in line with an inverse correlation of receptor activity and APP processing rates in cultured cells (3). However, the molecular...
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**FIGURE 1. SORLA deficiency accelerates APP processing in mice.** A, Western blot analysis of hippocampal extracts from 6-month-old wild type (+/+) and SORLA-deficient mice (−/−) indicates the absence of SORLA expression in knockouts (upper panel), accompanied by a reduction in full-length mature and immature murine APP (bracket, middle panel, IgG 1227), as well as an increase in soluble (s) APP levels (lower panel, IgG 22C11). B, detection of SORLA in perinuclear vesicles (arrows) of primary hippocampal neurons from wild type (+/+) but not from knockout mice (−/−, inset). C, reduction in mature and immature human APP (bracket, upper panel, IgG 1227) but increases in sAPP (middle panel, IgG WO2) levels in primary hippocampal neurons from Sora−/− compared with Sora+/+ mice. Immunodetection of tubulin is shown as loading control (lower panel). D, quantification of sAPP levels in hippocampal neurons of the indicated Sora genotypes as determined by densitometric scanning of Western blots exemplified in C (n = 6 independent experiments, IgG 22C11). E, immunohistological detection of SORLA in the pyramidal cell layer of the hippocampus. The panel to the right shows a high power magnification of the indicated area (CA2 region), in the left panel (F) Nissl-stained histological sections of the hippocampus from Sora−/− and Sora+/+ mice. mat, mature APP; im, immature APP.

mechanism underlying accelerated APP processing in this mouse model, as well as its effect on neuronal activity and survival remained an open issue. Here, we have investigated in detail the consequences of the loss of SORLA activity in vivo, particularly focusing on the hippocampus, a region in the brain that is especially vulnerable to AD-related disease processes.

When APP was detected in hippocampal extracts from Sora−/− mice using Western blot analysis, immunoreactive bands corresponding to the full-length forms of APP were specifically reduced compared with control animals (Fig. 1A, middle panel). The loss of APP protein was more pronounced for the fully glycosylated mature than the immature polypeptide variant. Thus, this decrease in mature APP mass in SORLA knockout mice may have been missed in previous studies where the bands corresponding to mature and immature APPs, respectively, were not resolved by SDS-PAGE (3). The reduction in APP was likely caused by accelerated proteolytic processing as indicated by a concomitant increase in soluble (s) APP products in receptor-deficient tissue (Fig. 1A, lower panel). Similar findings were obtained in primary cultures of hippocampal neurons where expression of SORLA can be seen in the perinuclear region in cells from wild type but not from SORLA-deficient newborn mice (Fig. 1B). Again, loss of receptor activity in knockout cells resulted in a distinct decrease in full-length APP and a massive increase in soluble APP-processing products (Fig. 1C). Using densitometric scanning of Western blots exemplified in Fig. 1C, the amount of mature APP was calculated to be reduced by ~70% (data not shown) whereas sAPP was increased by more than 50% (Fig. 1D) as compared with wild-type neurons. Accelerated conversion of APP into its processing products was also confirmed in an independent mouse model of SORLA deficiency generated by insertion of a β-galactosidase reporter gene into the murine Sora gene coding region (Fig. 2) (sorEX255, kindly provided by W. C. Skarnes). No changes in App gene transcription rates were detected by gene expression profiling comparing Sora+/+ and Sora−/−-deficient brain tissues (data not shown). Alterations in APP processing in Sora−/− animals did neither affect the overall anatomy of the hippocampus (Fig. 1F), a site of high receptor expression (Fig. 1E); nor other regions of the CNS (data not shown).

To also explore the consequence of insufficient SORLA activity for amyloidogenic processes and senile plaque formation, we introduced the Sora gene defect into the PDAPP line of mice that carries the human AppV717F transgene. This line represents a well-established mouse model of AD pathology (14). In hippocampal neurons from newborn mice doubly transgenic for human AppV717F and the Sora null allele, levels of full-length human APP were reduced (Fig. 3A) but concentrations of human sAPPα (Fig. 3, A and B), sAPPβ (Fig. 3, A and C), and Aβ40 (Fig. 3D) were significantly increased compared with PDAPP mice expressing SORLA. These findings provide in vivo confirmation of previous reports that demonstrated an inhibitory effect of SORLA activity on both α- and β-secretase activities in CHO cells (15); an inhibitory activity lost in SORLA-deficient neurons. Consistent with a proposed role for SORLA in retention of APP in the Golgi, the total amount of precursor molecules specifically in this organelle was reduced in neurons from (PDAPP, Sora−/−) mice compared with (PDAPP, Sora+/+) littermates as shown by immuno-electronmicroscopy (Fig. 4).

In the PDAPP line of mice, amyloid deposition starts around 6–7 months of age and reaches appreciable levels around 10 months (16). The extent of human Aβ production in the PDAPP line was significantly accelerated when the Sora gene defect was introduced into this model (Fig. 5A), resulting in a 3-fold increased plaque burden (Fig. 5B). Enhanced plaque deposition was seen in all brain areas but was particularly prominent in the hippocampus (Fig. 5C). Similar findings of increased
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SORLA deficiency enhances APP processing in the sorEX255 line of mice. A, Western blot analysis of total brain extracts (left panel) from wild type (+/+) and SORLA-deficient mice (−/−) of the sorEX255 line of mice indicates the absence of SORLA expression in knockouts (panel SORLA), accompanied by a reduction in full-length APP (bracket, panel APP, IgG 1227), and an increase in soluble (s) APP levels (panel sAPP, IgG 22C11). As a loading control, the membranes were reprobed with an antibody directed against neuron-specific β-tubulin. The asterisk in panel APP indicates chondroitin sulfate proteoglycan-modified APP molecules that are also reduced in Sorla−/− animals. A similar increase in sAPP levels was seen in extracts of primary hippocampal neurons from sorEX255 mice compared with control animals (right panel, IgG 22C11). B, quantification of mature (m) APP levels in primary hippocampal lysates of wild type and SORLA-deficient sorEX255 mice (set at 100%) determined by densitometric scanning of Western blots as exemplified in A (n = 4 independent experiments, IgG 1227).

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SORLA deficiency enhances APP processing in the PDAPP mouse. A, Western blot analysis of hippocampal neurons from PDAPP mice either wild type (+/+) or genetically deficient (−/−) for Sorla indicates decreased levels of full-length human APP (upper panel, IgG 1227) but increased levels of sAPPα and sAPPβ (lower panels) in mice lacking the receptor. As control, a neuronal extract from a non-APP-transgenic mouse was evaluated in parallel (ctr) to indicate immunoreactive bands corresponding to endogenous murine APP. Bands corresponding to mature and immature forms of human APP were confirmed by immunohistology in primary hippocampal neurons of PDAPP mice with the indicated Sorla genotypes as determined by densitometric scanning of Western blots exemplified in A (n = 5 independent experiments). B and C, quantification of sAPPα (IgG W02) and sAPPβ (IgG JP18957) in primary hippocampal neurons of PDAPP mice with the indicated Sorla genotypes as determined by densitometric scanning of Western blots exemplified in A (n = 5 independent experiments). D, ELISA indicates a 73% increase in human Aβ40 levels in hippocampal neurons from PDAPP mice lacking Sorla (−/−) compared with control animals heterozygous for the receptor gene defect (+/−, set at 100%) (n = 12 in each genotype).

plaque deposition were also obtained when the sorEX255 model of receptor deficiency was introduced into the PDAPP line (supplemental Fig. S1). Taken together, these data substantiated a role for SORLA in protection of APP from secretase processing in vivo, and the significance of low receptor activity as a cause of enhanced precursor breakdown and senile plaque formation.

Similar to other murine models of AD, aggravated plaque load did not significantly affect overall neuroanatomy in (PDAPP, Sorla−/−) mice as determined by immunohistology for markers of cholinergic neurons, activated microglia, and astroglia (supplemental Fig. S2), or apoptotic nuclei (data not shown).

To test whether increased Aβ levels in SORLA-deficient animals may impair neuronal activity we determined basis synaptic transmission and LTP in the CA1 area of the hippocampus. In agreement with previous reports (17), the presence of the App695transgene per se resulted in impairment in synaptic transmission as indicated by a decrease in the slope of input-output curves when comparing Sorla+/− (Fig. 6A, open circles) with PDAPP, Sorla+/− (open squares, p < 0.05) animals. In contrast, LTP was not altered by human APP expression (comparing data for Sorla+/− animals in Fig. 6A, quantification of sAPP). Similar to other murine models of AD, increased Aβ oligomers and senile plaques as causes of neuronal dysfunction and ultimate cell death in AD patients is firmly established, it remains debatable how soluble APP products influence normal neuronal function and how they may affect AD disease processes. In particular, sAPP was shown to act as a physiological signaling factor in MAP kinase pathways that are likely to be changed in the con-
text of altered APP-processing fates (18). Using Western blot analysis, we, therefore, investigated the consequences of increased sAPP levels in primary hippocampal neurons from wild type and SORLA-deficient newborn mice. We focused on the activity of ERK, a MAP kinase proposed to act downstream of sAPP in signaling processes in neurons (19). Intriguingly, a

FIGURE 4. Reduced number of APP molecules in the Golgi of Sorla-deficient neurons. A, quantification of APP immunoreactivity in the Golgi region of pyramidal neurons in PDAPP mice of the indicated Sorla genotypes using immuno-electronmicroscopy (IgG1227). B, micrograph shows one example of a total of 33 Golgi fields from each genotype that were used to determine the number of gold grains (arrows) corresponding to APP per nm² Golgi area in A. C, Western blot analysis of APP and tubulin (loading control) in brain extracts from two App⁺/⁻ and two App⁻/⁻ mice to document specificity of the anti-APP antiserum 1227 used in B.

FIGURE 5. Increased plaque deposition in PDAPP mice lacking SORLA. A, amount of insoluble Aβ extracted from brains of 7- and 10-month-old PDAPP mice either +/− or −/− for Sorla was determined by densitometric scanning of Western blots (inset, IgG MX-02) and depicted as relative Aβ levels normalized to actin (n = 5–10 animals per genotype). Because data failed the normality test, Aβ determinations were compared using the Manne-Whitney U test. B, amyloid plaque burden in 10-month-old PDAPP mice either +/− (n = 13) or −/− for Sorla (n = 9) was evaluated by immunostaining for human Aβ in the subiculum and hippocampus, and expressed as relative plaque load compared with the controls (set at 100%). The significance of the data was shown by Mann-Whitney U test. C, immunostaining for plaques (IgG 4G8) in the hippocampal region of two heterozygous and two SORLA-deficient mice (10 months of age).

FIGURE 6. Electrophysiological analysis of SORLA-deficient mice. Input-output curves for basal synaptic transmission (A) and long-term potentiation (B and C) in area CA1 of the hippocampus of mice of the indicated genotypes are shown (age: 10–12 months, n = 6–11 for each genotype). The presence of the human APP transgene causes a decrease in the slope of the input-output curves both in PDAPP, Sorla⁻/⁻ (open squares, p < 0.05) and PDAPP, Sorla⁻/⁻ (closed squares, p < 0.001) compared with respective controls (A). Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparison test. No change in LTP is seen in the presence of the Sorla gene defect on the background of murine (B) and human APP (C).
immature neurons (positive for DCX) (Fig. 8/H11011).

90% of surviving cells after 4 weeks were post-mitotic mature neurons (positive for NeuN) (Fig. 8D). No discernable difference in the percent distribution between neuronal and non-neuronal (GFAP-positive) cell populations was seen between the Sorla genotypes (data not shown). Enhanced neurogenesis in Sorla−/− animals was exclusive and not accompanied by increased neuronal cell death as tested by TUNEL on hippocampal sections (Fig. 9A) or Western blot for activated caspase 3 in primary hippocampal neurons (Fig. 9B).

Potentially, stimulation of ERK signaling and enhancement of neurogenesis in Sorla−/− animals may not be linked to altered APP processing but caused by lack of a yet unknown SORLA activity unrelated to APP metabolism. To exclude this possibility, we introduced the receptor gene defect into an APP-deficient mouse line (24) and investigated the consequences for neuronal signaling and proliferation in this new animal model. No APP-processing products, such as sAPP, can be detected in SORLA-deficient mouse (Fig. 10A, inset). Consistent with our hypothesis, neuronal survival 4 weeks after BrdUrd injection was identical in APP-deficient mice with or without Sorla gene defect (Fig. 10A).

Furthermore, a specific increase in ERK phosphorylation (pERK) in hippocampal extracts was only seen in (App+/−, Sorla−/−) animals (Fig. 10B, lane 2), but not in lines that lacked APP expression (Fig. 10B, lanes 3 and 4). Similarly, no elevated activity in this mouse model, we detected a 44% increase in ERK1 and a 102% increase in ERK2 phosphorylation in SORLA-deficient hippocampi compared with wild-type controls (data not shown). Activity levels of other kinases, such as glycogen synthase-serine kinase 3α, stress-activated protein kinase, c-Jun N-terminal kinase, as well as protein kinase C isofoms λ, ε, and γ were not altered in this screen (not shown).
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pERK levels were detected when primary hippocampal neurons from (App\(^{-/-}\), Sorla\(^{-/-}\)) were compared with those from (App\(^{-/-}\), Sorla\(^{+/+}\)) newborns (Fig. 10B, lanes 5 and 6). Thus, stimulation of ERK activation and enhancement of neurogenesis in mice lacking SORLA is dependent on the presence of APP and/or its processing products. The APP-processing product in question is likely to be soluble APP and not A\(_\beta\) because sAPP\(_\alpha\) caused a concentration-dependent increase in phosphorylation of ERK when applied to primary neuronal cultures. In contrast, A\(_\beta\) had no effect (Fig. 11). These findings are in agreement with previous reports (25, 26).

In conclusion, our findings have established an important contribution of SORLA activity to neuronal processing of APP in vivo; an activity that not only shows relevance for amyloidogenic processing and senile plaque formation, but that may also be crucial for control of ERK signaling pathways and for the regulation of adult neurogenesis.

**DISCUSSION**

Consistent with previous hypotheses obtained in cultured cells (3, 4, 6), we now have substantiated the role for SORLA as a regulator of APP processing in the murine brain. Loss of receptor activity in different mouse models results in accelerated turnover of full-length APP into its processing products, and in a significant increase in amyloid peptide formation. As well as for endogenous murine APP, such a regulatory role for SORLA has now also been documented for a human APP variant expressed in SORLA-deficient mice. These findings highlight the functional conservation of SORLA activities in rodent models and in humans, and they provide support for the concept that insufficient SORLA expression in patients may represent a risk factor for human AD (8). A reduction in synaptic number (input-output curves) but no change in synaptic strength (LTP) has been demonstrated in the PDAPP model before, pointing to some of the limitations of this and other APP transgenic mouse models in terms of human AD pathology (17, 27). Thus, plaque load in murine models is generally not directly correlated with neuronal deficits; an assumption supported by normal LTP in the PDAPP, Sorla\(^{-/-}\) animals despite a 3-fold increase in plaque burden compared with PDAPP, Sorla\(^{+/+}\) controls.

Assuming a role for SORLA in regulation of APP processing, one wonders what the physiological relevance of such receptor activities may be? This question is particularly relevant given the fact that the absence of the receptor (as in Sorla knockout mice) not only results in an increase in A\(_\beta\) but also in a dramatic rise in soluble APP products. Similar changes can be envisioned in AD patients suffering from low...
levels of receptor expression. SORLA-deficient mice described in this study enabled us to address the relevance of SORLA activity for sAPP production, and the consequences for neuronal function.

Because of a proposed mitogenic activity of sAPP, we evaluated proliferation of cells in the adult mouse brain using BrdUrd incorporation. In line with high sAPP levels in SORLA-deficient mice, we observed a strong increase in the number of newborn cells in the adult brain of these animals. Because sAPP was previously shown to enhance differentiation of progenitors into astrocytes (28) and to stimulate neural progenitor proliferation (29), we determined the fraction of proliferating cells being of glial or neuronal origin using co-staining of BrdUrd with marker proteins NeuN, DCX, and GFAP. In these experiments, a specific increase in the fraction of newborn neurons in the dentate gyrus was detected. Because both the rate of cell proliferation and survival were altered to a similar extent, we conclude that SORLA deficiency exerts its primary effect at the level of precursor cell proliferation.

In the adult brain, there are two major neurogenic regions; the subgranular zone of the dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles. Recently, evidence for a role of soluble APP products in adult neurogenesis was provided by Caillé et al. (29) who demonstrated a sAPP-dependent increase in the proliferation of SVZ progenitor cells when recombinant sAPPα was infused into the lateral ventricle. Because no sAPP binding was detected for cells of the hippocampus, it was suggested that soluble APP products exert their mitogenic stimulus predominantly in the SVZ (29). This conclusion contrasts with observations in this study that identified an increase in precursor cell proliferation in the hippocampus of Sorla knockout mice, most likely as a consequence of high local SAPP concentrations.

Another pathophysiological consequence of SORLA deficiency uncovered in this study was the aberrant activation of ERK signaling pathway in hippocampal neurons of receptor-null mice. In this model, ERK activation was critically dependent on the wild-type App gene, indicating that signaling proceeds through APP or its processing products. The most likely candidate for a signaling factor derived from the APP gene locus again is sAPP that is known to stimulate MAP kinase signaling. In cultured cells, stimulation of several kinase pathways, such as ERK and AKT, have been documented (19, 20, 21). Based on screens performed in brain and primary hippocampal neurons of Sorla−/− animals here, ERK seems to be the neuronal pathway most relevant in the hippocampus in vivo.

Some studies have linked ERK signaling to neural precursor cell proliferation. Although a direct correlation between ERK signaling and precursor cell proliferation has not formally been established in this study, it is tempting to speculate, that over-activity of the ERK pathway in the SORLA-deficient mouse is the cause of enhanced adult neurogenesis observed in this model. Intriguingly, several reports have described increased cell proliferation and even adult neurogenesis in AD (30, 31). Signs of increased adult hippocampal neurogenesis were found in patients suffering from sporadic AD (30), but the specificity of the observation has been disputed (32). Similarly, in murine models both reports of up- and down-regulation of adult neurogenesis exist (30, 33, 34). Still, activity of ERK and its direct upstream kinase MEK1 is markedly increased in AD brains (35–38). The reason for a potentially enhanced neurogenesis in some AD patients has been unclear so far. Compensatory mechanisms whereby the diseased brain tries to cope with neuronal cell loss has been proposed as one of several models. Assuming that SORLA deficiency may be the underlying cause of AD pathology in some AD patients, our findings provide a molecular explanation for the latter hypothesis inasmuch as enhanced neurogenesis may be the consequence of increased sAPP levels as a result of receptor deficiency. In this respect, the Sorla knockout mouse represents an excellent model system with which to study the mechanisms underlying AD-associated ERK activation.

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