Brief Communications

EPAC Inhibition of SUR1 Receptor Increases Glutamate Release and Seizure Vulnerability

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EPAC (Exchange Proteins Activated by cAMP) regulates glutamate transmitter release in the central neurons, but a role underlying this regulation has yet to be identified. Here we show that EPAC binds directly to the intracellular loop of an ATP-sensitive potassium (K_{ATP}) channel type-1 sulfonylurea receptor (SUR1) receptor consisting of amino acids 859 – 881 ($SUR1^{859-881}$). Ablation of EPAC or expression of $SUR1^{859-881}$, which intercepts EPAC-SUR1 binding, increases the open probability of K_{ATP} channels consisting of the Kir6.1 subunit and SUR1. Opening of K_{ATP} channels inhibits glutamate release and reduces seizure vulnerability in adult mice. Therefore, EPAC interaction with SUR1 controls seizure susceptibility and possibly acts via regulation of glutamate release.

Introduction

EPAC (Exchange Proteins Activated by cAMP) belongs to a novel class of cAMP receptors (de Rooij et al., 1998; Bos, 2006). There are two isoforms of EPAC proteins and each has multiple domains, consisting of one (EPAC1) or two (EPAC2) cAMP regulatory binding motifs (Kawasaki et al., 1998; Rehmann et al., 2003). Both EPAC1 and EPAC2 proteins are expressed throughout the brain, including in the hippocampus, striatum, and prefrontal cortex (Zhang et al., 2009; Yang et al., 2012). To determine their neurological functions, we developed mutant strains of mice with deficiency in expression of either EPAC1 ($EPAC1^{-/-}$) or EPAC2 ($EPAC2^{-/-}$) or both ($EPAC^{-/-}$) genes (Yang et al., 2012). We showed previously that combined deletion of both the EPAC1 and EPAC2 genes ($EPAC^{-/-}$) reduces glutamate release from the presynaptic terminals (Yang et al., 2012).

Transmitter release involves several steps of interactions between synaptic and vesicle fusion proteins (Südhof, 1995; Schneggenburger and Neher, 2005; Haucke et al., 2011) and requires Ca^{2+} influx into the terminals (Stanley, 1997; Jackson and Chapman, 2008). Previously, we described ATP-sensitive potassium (K_{ATP}) channels consisting of Kir6.1 subunit and type-1 sulfonylurea receptor (SUR1) at the presynaptic terminals in the hippocampus (Soundarapandian et al., 2007). K_{ATP} channels are gated by metabolic factors such as ATP/ADP ratios (Ashcroft and Gribble, 1998; Schwappach et al., 2000). In pancreatic β-cells, K_{ATP} channels are associated directly with EPAC2 protein and thus control Ca^{2+}-dependent secretion of insulin (Zhang et al., 2009). However, whether EPAC interacts with K_{ATP} channels in the central neurons is not known.

In the present study, we used a gene-targeting approach combined with electrophysiological recordings to show that EPAC physically and functionally interacts with K_{ATP} channels via direct inhibition of the SUR1 receptor in the dentate granule cells. We found that this inhibition controls glutamate release and seizure vulnerability in adult mice.

Materials and Methods

Development of EPAC mutant mice. The conditional mutant strain of mice with a selective deletion of EPAC1 gene in the hippocampus ($EPAC1^{-/-}$ mice) was generated by gene targeting in 129Sv embryonic stem cells, as described previously (Yang et al., 2012). EPAC2-null mutant ($EPAC2^{-/-}$) mice were generated using a gene-trapping approach in 129Sv mouse embryonic stem cells (Yang et al., 2012). A double mutant strain of mice ($EPAC^{-/-}$ mice) was developed by crossing $EPAC1^{-/-}$ mice with $EPAC2^{-/-}$ mice. $SUR1^{-/-}$ mice were purchased from The Jackson Laboratory and bred with $EPAC^{-/-}$ mice, resulting in the $EPAC^{-/-}/SUR1^{-/-}$ mice. Care and experiments with animals were in accordance with institutional guidelines and those of the animal care and use committees of Huazhong University of Science and Technology (Wuhan, China) and the Louisiana State University Health Sciences Center (New Orleans).

Co-IP experiments. Synaptosomes were prepared as described previously (Peng et al., 2006; Soundarapandian et al., 2007). Briefly, the hippocampal homogenate in 0.32 M sucrose was centrifuged for 10 min at 1400 × *g* to yield a pellet (P1) and a supernatant (S1). S1 was centrifuged for another 10 min at 13,800 × *g*, yielding a crude synaptosomal pellet (P2) and a supernatant (S2). P2 was resuspended in 0.32 M sucrose con-

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taining 1 mM $NaHCO_3$ and layered on top of a discontinuous sucrose gradient (0.8, 1.0, and 1.2 M). After centrifugation for 2 h at 82,500 × *g*, the synaptosomes were recovered as a band, resuspended in 0.32 M sucrose and 1 mM $NaHCO_3$ plus protease inhibitors, pelleted, and resuspended in HEPES buffer containing protease inhibitors.

Synaptosomes were incubated with 1% Triton X-100 for 20 min on ice and centrifuged at 14,000 g for 15 min to obtain the supernatant. Protein concentration in the extracts was determined by Lowry assay (Bio-Rad). The extracts (~500 μg of protein) were incubated with polyclonal rabbit anti-EPAC1 (2 μg) or anti-EPAC2 (2 μg) overnight at 4°C, followed by the addition of 40 μl of Protein G-Sepharose (Sigma) for 3 h at 4°C. Immunoprecipitates were washed four times with PBS, denatured with SDS sample buffer, separated by SDS-PAGE, and blotted with anti-Kir6.1 (1:100; Santa Cruz Biotechnology), anti-SUR1, or anti-syntaxin-1A (1:400; Santa Cruz Biotechnology) antibodies.

Electrophysiology. The slices (350 μm) of the hippocampus were cut from male mice at 90 ± 5 d of age and placed in a holding chamber for at least 1 h. A single slice was then transferred to the recording chamber and submerged and perfused with artificial CSF (2 ml/min) that had been saturated with 95% O_2/5% CO_2. The composition of the artificial CSF was as follows (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Whole-cell patch–clamp recordings (5 MΩ) at voltage–clamp mode were visualized with infrared differential interference contrast using an Axioskop 2FS equipped with Hamamatsu C2400–07E optics, as described previously (Peng et al., 2006; Tu et al., 2010; Yang et al., 2012).

The internal pipette solution for whole-cell patch–clamp recordings contained the following (in mM): 132.5 cesium gluconate, 17.5 CsCl, 0.05 EGTA, 10 HEPES, 2 Mg-ATP, and 0.5 GTP, pH 7.4 (290 mOsm). NMDA receptor-mediated EPSCs were evoked by paired-pulse stimulation of the mossy fiber tracks in the hilus of the dentate gyrus using bipolar tungsten electrodes and recorded with Axopatch 200 B amplifiers and monitored by computer using *p*Clamp11 at 35°C in the presence of 10 μM bicuculline and 20 μM NBQX at a holding potential of +60 mV. The spontaneous EPSCs were recorded in the presence of 100 μM AP5 and 10 μM bicuculline at a holding potential of −70 mV. The threshold (~6 pA) for detection was set at 3× the baseline SD from a template of 0.5 ms rise time and 10 ms decay. Cells with a noisy or unstable baseline (5 min after break-in) were discarded.

For single-channel recordings from the dentate granule cell in the hippocampus, the pipette solution contained the following (in mM): 140 potassium methanesulfonate (KMeS), 10 HEPES, 2 $CaCl_2$, 10 TEA-Cl, 2 CsCl, 1 4-aminopyridine (4-AP), 100 nM charybdotoxin, and 100 nM aparmin, pH 7.4 (290 mOsm).

Generation of rAAV1/2 virus particles. SUR1$^{859-881}$ (dhlmqagilellrddkrtvvlvt) and its scrambled control (lmqdhllrdagilellvlvtkrt) were cloned into the rAVE construct containing eGFP through ApaI/KpnI (GenDetect), creating rAVE-CAP/ SUR1$^{859-881}$-IRES-eGFP vectors. The rAVE plasmids were cotransfected with the AAV helper1 and helper 2 into HEK293 cells to generate the rAAV1/2 virus particles. Generation of the infectious virus particles (>5 × 10^{12} genomic particle/ml) were described previously (Peng et al., 2006; Tu et al., 2010). Activated virus particles were coded by experimenters. Other experimenters, who were unaware of the coded particles, injected the particles (2 μl at 0.2 μl/min) into each side of the dorsal hippocampus (3.1 mm posterior to bregma; 2.3 mm lateral to the midline; 2.9 mm below dura).

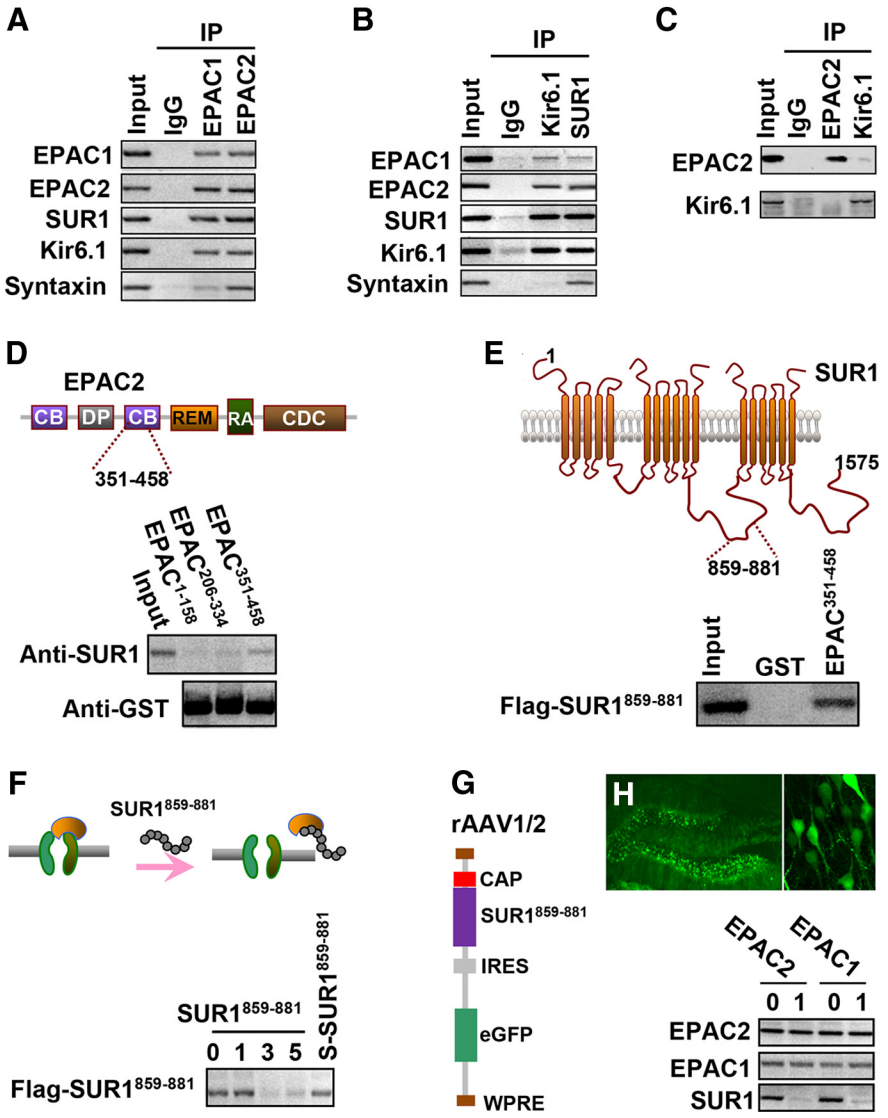

Figure 1. EPAC binds to SUR1 directly. ***A–C***, Synaptosomes were prepared from SUR1$^{+/+}$ mice (***A***,***B***) and SUR1$^{-/-}$ mice (***C***) and precipitated using antibodies against EPAC1 and EPAC2 or Kir6.1 and SUR1, as indicated. The precipitates were blotted with antibodies against EPAC1, EPAC2, SUR1, Kir6.1, and syntaxin-1A. Input: 20 μg of proteins without precipitation were loaded. Similar results were seen in each of the four experiments. ***D***, Synaptosomes were precipitated using GST-EPAC2$^{1-158}$, GST-EPAC2$^{206-334}$, or GST-EPAC2$^{351-458}$ and blotted with anti-SUR1. Input: 20 μg of protein without precipitation was blotted with antibodies against GST and SUR1, as indicated. Similar results were seen in each of the four experiments. ***E***, GST-EPAC2$^{351-458}$ or GST alone was coexpressed with Flag-tagged SUR1$^{859-881}$ in HEK293 cells. Then, 72 h after expression, cell lysates were precipitated using anti-GST and blotted with anti-Flag antibody. Input: 10 μg of proteins without precipitation were loaded. Similar results were seen in each of the four experiments. ***F***, GST-EPAC2$^{351-458}$ was coexpressed with Flag-tagged SUR1$^{859-881}$ in HEK293 cells. Then, 72 h after expression, cell lysates were precipitated using anti-GST in the presence of 5 μg/ml SUR1$^{859-881}$ peptide or its scrambled control (S-SUR1$^{859-881}$) and blotted with anti-Flag antibody. Similar results were seen in each of the five experiments. ***G***, ***H***, The rAAV1/2 vector (***G***) for expression of SUR1$^{859-881}$ and a representative image (***H***) of the dentate gyrus area taken 15 d after injection of the rAAV1/2-CAP/SUR1$^{859-881}$-IRES-eGFP virus particles. Synaptosomes were prepared from the dentate gyrus 15 d after expression of SUR1$^{859-881}$ (lane 1) or its scrambled control (lane 0) and precipitated with anti-EPAC1 and anti-EPAC2, respectively. The precipitates were blotted with antibodies against EPAC1, EPAC2, or SUR1, as indicated. Similar results were seen in each of the five experiments.

Kainic acid treatment and behaviors. Adult male mice (age 90 ± 5 d, 28 ± 2 g of body weight) were injected intraperitoneally with a single dose of kainic acid (KA; 25–40 mg/kg in PBS, A.G. Scientific). Mice were monitored continuously for 3 h. The severity of seizures was rated by the arbitrary scale, with 1 = staring and immobility/wet dog shake, 2 = hyperactivity, repetitive movements, rearing, and falling, 3 = low seizures (intense shivering), 4 = severe tonic/clonic convulsion, and 5 = death. The averaged points for seizure severity in a given group were expressed as the seizure index, as described previously (Soundarapandian et al., 2007).

Statistical analysis. Data were analyzed using SPSS 11.0 statistical software. All data are expressed as mean ± SEM. Statistical significance was determined by one-way ANOVA followed by a Student–Newman–Keuls *post hoc* test with 95% confidence and Student's two-tailed *t* test.

Results

EPAC2$^{351-458}$ binds to SUR1$^{859-881}$

To search for an interaction between EPAC and K_{ATP} channels, we precipitated the EPAC protein complex in the hippocampus of adult mice. Blots of the precipitates revealed that EPAC proteins were physically associated with the K_{ATP} channel components, including the Kir6.1 subunit and the SUR1 receptor (Fig. 1*A*,*B*). This association was eliminated in SUR1$^{-/-}$ mice (Fig. 1*C*), revealing that the SUR1 receptor acts as an intermediary between EPAC and Kir6.1 channels. As a positive control, we blotted the precipitates with anti-syntaxin-1A, a functional component of synaptic vesicle proteins that is known to interact with SUR1 receptor in the central neurons (Chang et al., 2011; Zhou et al., 2012). To validate this association, we generated a series of truncation mutants of SUR1 and EPAC proteins (Fig. 1*D*,*E*) and found that a GST-EPAC2 protein consisting of amino acids 351–458 (GST-EPAC2$^{351-458}$) was able to pull down endogenous SUR1 receptor in synaptosomes from the hippocampus of adult mice (Fig. 1*D*). Coexpression of GST-EPAC2$^{351-458}$ with Flag-SUR1$^{859-881}$ in HEK293 cells revealed that the EPAC2 protein bound directly to the SUR1 receptor (Fig. 1*E*). Direct binding between GST-EPAC2$^{351-458}$ and Flag-SUR1$^{859-881}$ was verified by a peptide-blocking experiment (Fig. 1*F*) in which a synthesized SUR1$^{859-881}$ peptide at a concentration of 5 μg/ml sufficiently inhibited an association of GST-EPAC2$^{351-458}$ with Flag-SUR1$^{859-881}$. To examine whether SUR1$^{859-881}$ intercepts EPAC association with SUR1 in brain cells *in vivo*, we expressed SUR1$^{859-881}$ in the hippocampus of adult mice using the rAAV1/2 virus vector (Fig. 1*G*). Fifteen days after expression, we precipitated endogenous EPAC proteins. Western blots of the precipitates revealed that SUR1$^{859-881}$ uncoupled EPAC proteins from SUR1 receptor in the hippocampus (Fig. 1*H*), demonstrating that EPAC is physically associated with K_{ATP} channels via direct binding between EPAC2$^{351-458}$ and SUR1$^{859-881}$.

Increased open probability of K_{ATP} channels in EPAC$^{-/-}$ mice

K_{ATP} channels consist of inwardly rectifying K^+ channels (Kir6.1, Kir6.2) and regulatory sulfonylurea receptors (SUR1, SUR2A, and SUR2B), which are members of the ATP-binding cassette proteins (Ashcroft and Gribble, 1998; Schwappach et al., 2000). A combination of the different subunits forms different types of K_{ATP} channels in different cell types. We showed previously that the presynaptic K_{ATP} channel consists of the Kir6.1 subunit and the SUR1 receptor in the hippocampus (Soundarapandian et al., 2007). Therefore, we investigated here whether EPAC regulates the Kir6.1/SUR1 type of K_{ATP} channels functionally. We performed single-channel recordings in the dentate granule cells of EPAC$^{-/-}$ mice and compared them with EPAC$^{+/+}$ mice (Fig. 2*A*). Open- and closed-channel distributions are shown in Figure 2*B*, *C*. We found that genetic deletion of EPAC genes increased open probability (P_{open}) of K_{ATP} channels. This increase was absent in both SUR1$^{-/-}$ and Kir6.1$^{-/-}$ mice (Fig. 2*D*). An increase of P_{open} was also seen in the wild-type mice expressing SUR1$^{859-881}$ (Fig. 2*E*), indicating that EPAC proteins regulate K_{ATP} channel open probability via a direct inhibition of the SUR1 receptor.

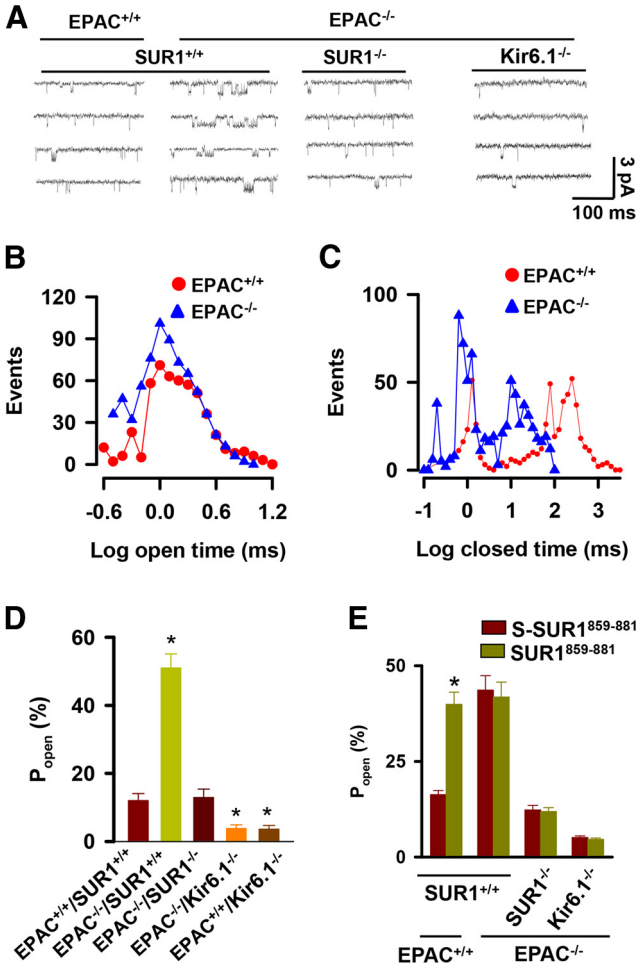

Figure 2. Deletion EPAC increases K_{ATP} channel open probability. ***A***, Traces are representative single-channel recordings from the dentate granule cells. Downward deflections of the current traces indicate single-channel openings. ***B***, ***C***, Graphs of open (***B***) and closed (***C***) time distributions of single K_{ATP} channel currents. ***D***, Bar graph summarizes single-channel currents in cell-attached patches from the dentate granule neurons of EPAC$^{+/+}$/SUR1$^{+/+}$, EPAC$^{-/-}$/SUR1$^{+/+}$, and EPAC$^{-/-}$/SUR1$^{-/-}$ mice. Data are mean ± SEM ($n = 8$ recordings/4 mice/genotypes, $^*p < 0.01$). ***E***, Interception of EPAC–SUR1 binding increases K_{ATP} channel open probability. The K_{ATP} single-channel currents were recorded from the dentate granule cells 15 d after expression of SUR1$^{859-881}$ or its scrambled control. Data are mean ± SEM ($n = 8$ recordings/4 mice/genotypes, $^*p < 0.01$).

Reduced glutamate release probability in EPAC$^{-/-}$ mice

Our prior studies showed that SUR1 receptor is associated with synaptic vesicle proteins at the presynaptic terminals (Soundarapandian et al., 2007). We thus investigated here whether an increase of K_{ATP} channel open probability in EPAC$^{-/-}$ mice alters glutamate transmitter release. We first analyzed the spontaneous EPSCs and found that their frequency was reduced in EPAC$^{-/-}$ mice compared with wild-type controls, whereas the mean amplitude did not differ between genotypes (Fig. 3*A*–*D*). We next recorded the paired-pulse facilitation of the evoked EPSCs at mossy fiber-CA3 synapses. To measure glutamate release from

the mossy-fiber terminal accurately and to avoid the polysynaptic responses within CA3-CA3 synaptic inputs, we recorded NMDA-receptor-mediated EPSCs in the presence of 20 μM NBQX. We found that EPAC$^{-/-}$ mice displayed greater paired-pulse facilitation than wild-type controls, with the greatest effects at the shortest interstimulus interval (Fig. 3*E*,*F*). This facilitation increased with an elevation of Ca^{2+} (Fig. 3*G*). Therefore, EPAC$^{-/-}$ mice have a reduction of Ca^{2+}-dependent glutamate release probability at the mossy-fiber terminals. The similar reduction of glutamate release was achieved by interception of EPAC-SUR1 association via expressing SUR1$^{859-881}$ peptide. Therefore, an interaction between EPAC and the SUR1 receptor regulates Ca^{2+}-dependent glutamate release.

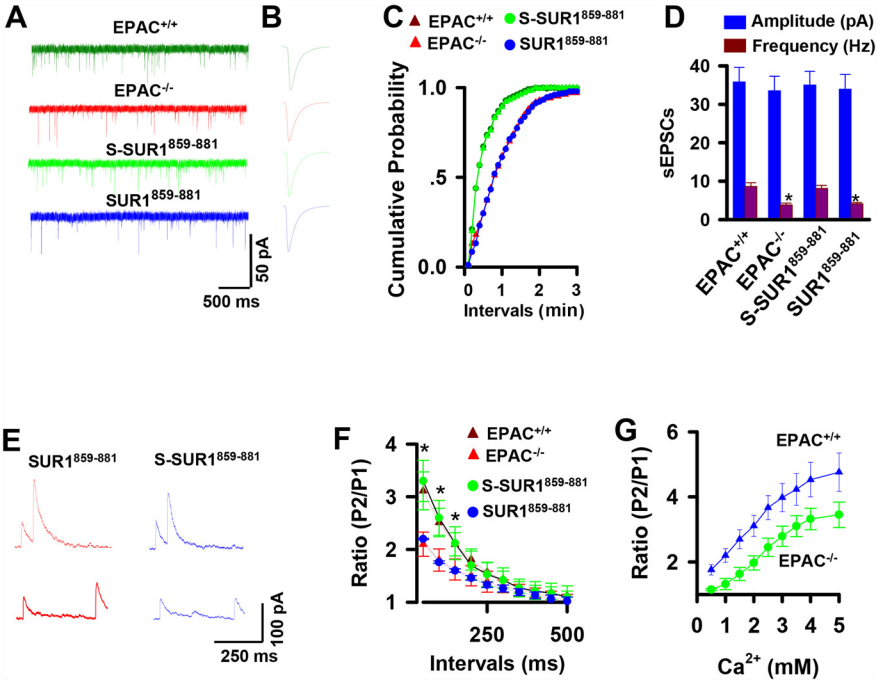

Figure 3. EPAC inhibition of SUR1 reduces glutamate release. ***A–D***, Representative spontaneous EPSCs (***A***), averaged responses (***B***), cumulative probability (***C***), and average frequency and amplitude (***D***) in CA3 pyramidal neurons of EPAC$^{+/+}$, EPAC$^{-/-}$, and EPAC$^{+/+}$ mice expressing SUR1$^{859-881}$ or scrambled SUR1$^{859-881}$. Data are mean ± SEM (n = 12 recording/6 mice per genotype, *$p < 0.01$). All recordings are at a hold potential of −70 mV. ***E***, Representative NMDA-receptor-mediated EPSCs from CA3 pyramidal neurons at a holding potential of +60 mV in the presence of 20 μM NBQX and 10 μM bicuculline are evoked by paired pulse stimulation of the mossy-fiber tracks with the interstimulus intervals of 50 ms (top) and 500 ms (bottom), respectively. ***F***, The paired-pulse ratios versus interstimulus intervals from EPAC$^{+/+}$ (brown triangles), EPAC$^{-/-}$ (red triangles), and EPAC$^{+/+}$ mice expressing SUR1$^{859-881}$ (blue circles) or scrambled SUR1$^{859-881}$ (green circles). Data are mean ± SEM (n = 12 recordings/6 mice/genotype, *$p < 0.01$). ***G***, The paired-pulse ratios increase with elevation of extracellular Ca^{2+} in EPAC$^{+/+}$ (blue triangles) and EPAC$^{-/-}$ (green circles). Data are mean ± SEM (n = 9 recordings/3 mice/genotype).

Reduced seizure vulnerability in EPAC$^{-/-}$ mice

Our earlier study revealed that, compared with the wild-type controls, mutant mice lacking the SUR1 gene (SUR1$^{-/-}$) or the Kir6.1 gene (Kir6.1$^{-/-}$) are vulnerable to epileptic seizures (Soundarapandian et al., 2007), a severe neurological disorder that is known to be associated with an increased glutamate release (Ben-Ari and Cossart, 2000). We thus hypothesized that an increase of K_{ATP} channel open probability in EPAC$^{-/-}$ mice reduces seizure sensitivity. To test this hypothesis, we examined adult mice for seizure activity after KA administration. The latency and severity of seizures were diagnosed and expressed as a seizure index with increased KA doses (Fig. 4*A*). We found that the majority of EPAC$^{+/+}$ mice (96%, n = 15 mice) underwent status epileptic seizures such as hyperactivity, constant rearing, and falling. Eleven of 15 mice exhibited tonic convulsion and died within 3 h when a single dose of 40 mg/kg was administered (Fig. 4*A*). When a single dose of 30 mg/kg was administered, the seizure index of EPAC$^{+/+}$ mice was of 3.62 ± 0.51 (mean ± SEM, n = 15, Fig. 4*B*). In striking contrast, few EPAC$^{-/-}$ mice progressed in severity to the extent of EPAC$^{+/+}$ mice; 10 of 11 EPAC$^{-/-}$ mice had no convulsive responses and remained alive throughout the course of observations with a seizure index of 1.38 ± 0.16 (mean ± SEM, n = 11). The similar reduction of epileptic seizures was observed in wild-type mice expressing SUR1$^{859-881}$ (Fig. 4*B*). Therefore, EPAC protein confers seizure vulnerability through direct inhibition of the SUR1 receptor.

Discussion

In our previous studies (Soundarapandian et al., 2007), we reported that a combination of the Kir6.1 subunit with the SUR1 receptor form neuronal-type K_{ATP} channels that are located predominantly at the presynaptic terminals. Genetic inhibition of either the Kir6.1 subunit or the SUR1 receptor enhances glutamate transmitter release, leading to the induction of epileptic seizures in adult mice (Soundarapandian et al., 2007). In the present study, we identified EPAC proteins as the functional interactive components of the Kir6.1/SUR1 type of K_{ATP} channels in the dentate granule cells. Genetic deletion of EPAC genes or disruption of EPAC-SUR1 interaction increased the open probability of K_{ATP} channels substantially, thus counteracting epileptic seizures occurring in the SUR1$^{-/-}$ mice. This finding indicates that EPAC controls K_{ATP} channel activity via tonic inhibition of the SUR1 receptor at the presynaptic terminals, as illustrated in Figure 4*C*, *D*.

Previous studies using pharmacological reagents showed that the EPAC protein regulates vesicular release probability in the central neurons (Sakaba and Neher, 2003; Zhong and Zucker, 2005), but the mechanism underlying this regulation remained unknown. In the present study, we have shown that EPAC proteins are associated with K_{ATP} channels via a direct binding between EPAC2 and the SUR1 receptor. K_{ATP} channels are gated by intracellular metabolic factors and opening of the channels hyperpolarizes cells, leading to a reduction of Ca^{2+}-dependent transmitter release (Fig. 4*C*). An increase of intracellular Ca^{2+} could be caused by either Ca^{2+} influx from voltage-dependent Ca^{2+} channels or Ca^{2+} release from the intracellular stores (Collin et al., 2005; Sharma et al., 2008). Deletion of EPAC genes or the interception of EPAC-SUR1 binding increases the open probability of K_{ATP} channels in the dentate granule neurons (Fig. 4*D*). Therefore, it is plausible that EPAC proteins control transmitter release at the granule cell terminals via a direct inhibition of the SUR1 receptor.

Several rare, nonsynonymous variants of EPAC genes have been reported in patients with autism spectrum disorders (Bacchelli et al., 2003), but whether these mutations cause autistic

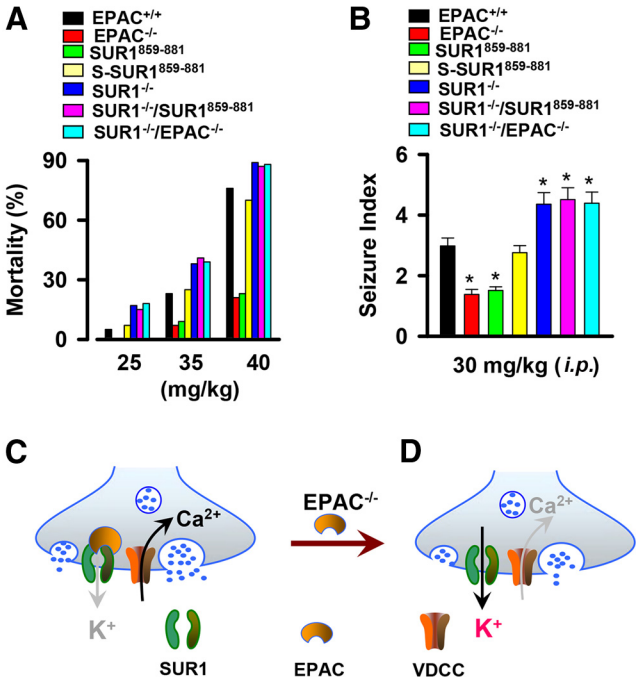

Figure 4. EPAC $^{-/-}$ mice have decreased vulnerability to epileptic seizures. ***A***, Bar graph showing the mortality rate due to convulsive responses within 3 h after intraperitoneal injection of KA at the doses from 25 to 40 mg/kg. Data are mean ± SEM ($n = 13$ mice/genotype). ***B***, Bar graph showing seizure index after a single intraperitoneal dose of 30 mg/kg. Data are mean ± SEM ($n = 13$ mice/genotype). ***C***, ***D***, Working model: EPAC controls glutamate release via tonic inhibition of SUR1 receptor at the presynaptic terminals. Opening of Kir6.1/SUR1 channels hyperpolarizes cells, leading to a reduction of Ca^{2+}-dependent transmitter release (***C***). Deletion of EPAC genes increases the open probability of K_{ATP} channels in the dentate granule neurons and reduces Ca^{2+}-dependent glutamate release (***D***), thereby antagonizing epileptic seizures.

behaviors remains unknown (Woolfrey et al., 2009). Autism patients are diagnosed with abnormalities of social interactions and mental retardation (Geschwind and Levitt, 2007). Some patients also exhibit spontaneous epileptic seizures (Walsh et al., 2008). Recently, we demonstrated that the EPAC mutation causes defects in spatial learning and social interactions (Yang et al., 2012). In the present study, we have also shown that the EPAC mutation elevates seizure vulnerability. We conclude that disruption of EPAC signaling may represent a molecular mechanism underlying the expression of autistic phenotypes.

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