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Forebrain-Specific Inactivation of G_q/G_{11} Family G Proteins Results in Age-Dependent Epilepsy and Impaired Endocannabinoid Formation†

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Metabotropic receptors coupled to G_q/G_{11} family G proteins critically contribute to nervous system functions by modulating synaptic transmission, often facilitating excitation. We investigated the role of G_q/G_{11} family G proteins in the regulation of neuronal excitability in mice that selectively lack the α -subunits of G_q and G_{11} , $G\alpha_q$ and $G\alpha_{11}$, respectively, in forebrain principal neurons. Surprisingly, mutant mice exhibited increased seizure susceptibility, and the activation of neuroprotective mechanisms was impaired. We found that endocannabinoid levels were reduced under both basal and excitotoxic conditions and that increased susceptibility to kainic acid could be normalized by the enhancement of endocannabinoid levels with an endocannabinoid reuptake inhibitor, while the competitive cannabinoid type 1 receptor antagonist SR141716A did not cause further aggravation. These findings indicate that G_q/G_{11} family G proteins negatively regulate neuronal excitability in vivo and suggest that impaired endocannabinoid formation in the absence of G_q/G_{11} contributes to this phenotype.

Several metabotropic receptors of the central nervous system signal through heterotrimeric G proteins of the G_q/G_{11} family, for example, the metabotropic glutamate receptor subtypes 1 and 5, the M_1 muscarinic acetylcholine receptor, and the 5-hydroxytryptamine 2 (5-HT₂) serotonin receptor. Stimulation of G_q/G_{11} -coupled receptors causes the activation of phospholipase C- β (PLC- β) isoforms, leading to inositol phospholipid breakdown, protein kinase C activation, and intracellular calcium mobilization (18). G_q/G_{11} -coupled receptors have been shown to contribute to the modulation of synaptic transmission in the hippocampus and cerebellum (1, 23, 25, 33, 37, 46, 48) and are known to facilitate neuronal activation by opening voltage-dependent cation channels and closing potassium channels (10, 12). In line with this, agonists at the G_q/G_{11} -coupled metabotropic glutamate receptors or the muscarinic acetylcholine receptors promote seizures in vitro and in

vivo (6, 11, 57), while antagonists at these receptors have been suggested to have anticonvulsive effects (7, 8).

In vitro data, however, indicate that G_q/G_{11} -coupled receptors can also indirectly exert inhibitory effects through the inhibition of presynaptic calcium channels (22, 45) or the activation of the endocannabinoid system. Endocannabinoids such as anandamide and 2-arachidonoyl glycerol (2-AG) are lipophilic neuromodulators that are synthesized and released “on demand” in postsynaptic neurons in response to depolarization-induced calcium influx (14, 34, 50, 69) or activation of G_q/G_{11} -coupled metabotropic receptors (20, 21, 32, 38, 41, 51, 52, 65). Both transmembrane Ca^{2+} influx and G_q/G_{11} -mediated intracellular Ca^{2+} mobilization enhance anandamide biosynthesis via an *N*-acyltransferase and a specific phospholipase D-like enzyme (13, 15, 16, 56, 61, 62). In parallel, G_q/G_{11} -mediated PLC- β activation results in the production of diacylglycerol (DAG), which serves as a precursor for 2-AG via Ca^{2+} -sensitive *sn*-1-DAG lipases (3). PLC- β , DAG, and *sn*-1-DAG lipase were suggested to be of special importance for the production of that pool of 2-AG that functions as an endocannabinoid precursor, especially in response to neuronal depolarization and in retrograde signaling (9, 16, 44, 56, 62, 63). Endocannabinoids act mostly via presynaptic cannabinoid type 1 receptors to inhibit neurotransmitter release at inhibitory as well as excitatory synapses (2, 19, 68), and they were shown to protect the brain from excitotoxicity and other forms of neuronal damage (16, 43, 56, 62, 63). However, the relative weight and function of G_q/G_{11} -mediated signaling in the regulation of endocannabinoid levels in vivo are unclear. It is also not clear whether the net effect of G_q/G_{11} signaling in vivo is excitatory or inhibitory.

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The two main members of the G_q/G₁₁ family are G_q and G₁₁, which are almost ubiquitously expressed in the central nervous system and have highly redundant functions. Since genetic inactivation of both G_{α_q} and G_{α₁₁} causes embryonic lethality (49), we employed the *Cre/loxP* system to generate mice which allow tissue-specific inactivation of G_{α_q} in constitutively G_{α₁₁}-deficient mice (67). These mice were crossed with the *Camkcre4* mouse line (39), which causes recombination selectively in principal neurons of the forebrain (40). Forebrain-specific G_{α_q}/G_{α₁₁} double knockout (fb-G_{α_q}/G_{α₁₁}-DKO) mice were viable and fertile and showed normal basic sensory and motor abilities, but females were unable to raise normal maternal behavior toward their young (66). We used fb-G_{α_q}/G_{α₁₁}-DKO mice to investigate the role of G_q/G₁₁ in the regulation of neuronal excitability, with a special focus on the role of G_q/G₁₁ in endocannabinoid synthesis.

MATERIALS AND METHODS

Mice. Genotyping for the *gnaq*^{fllox} allele, the *gna11* wild-type and knockout alleles, and the Cre transgene was described previously (67). The primers used for detecting the *Camkcre4* transgene were 5'-TCTCCATTTGACTCAGGAG C-3' and 5'-AAAACGCTGGCGATCCCT-G-3'. Mice were housed under specific-pathogen-free conditions, and all animal experiments were performed in accordance with institutional animal care and use committee regulations. The genetic background of the mice was predominantly C57BL6/N (fourth-generation backcross), and nonlittermate C57BL6/N mice or littermates with the *Camkcre4*^{-/-} *gnaq*^{fl/fl} *gna11*^{wt/wt} genotype were used as controls. No significant differences were observed between the two control groups.

Animal experiments. Kainic acid (KA; Sigma, Deisenhofen, Germany) and pentylenetetrazole (PTZ; Sigma) were dissolved in saline and administered intraperitoneally (i.p.) at the indicated dosages. *N*-Piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolone-carboxamide (SR141716A; Cayman Chemical, Ann Arbor, MI) and OMDM-2 [(*R*)-*N*-oleoyl-(1'-hydroxybenzyl)-2'-ethanolamine] (synthesized as previously described [(54)] were dissolved in Tween 80-ethanol-0.9% saline (1:1:18) and injected i.p. 30 min before KA treatment at the concentrations indicated in the figure legends (for six to eight mice per group). Mice were monitored for 60 min, and responses were scored every 5 minutes by an observer blinded to the genotype, as described previously (24). Briefly, responses were scored as follows: 0, normal behavior; 1, rigid or slowed walking; 2, isolated jerks; 3, repetitive jerks; 4, forelimb clonus, isolated clonic jerks; 5, repetitive clonic jerks, mouse on its side with fore- and hindlimb clonus; 6, death. Video and electroencephalography (EEG) monitoring of mice were performed as described previously (35).

Histology. Mice were deeply anesthetized with pentobarbital, 100 mg/kg of body weight i.p., and perfused with 4% paraformaldehyde via the left cardiac ventricle. Their brains were postfixed overnight and then stored in 0.5% paraformaldehyde at 4°C. Fifty-micrometer vibratome sections were cut and incubated at 4°C with antibodies directed against the following proteins: *c-fos* protein (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA; diluted 1:20,000, incubated for 72 h); zif-268 (Santa Cruz Biotechnology; diluted 1:200, incubated for 72 h); G_{α_q}/G_{α₁₁} (sc-392; Santa Cruz Biotechnology; diluted 1:1,000, incubated for 16 h); GFAP (DAKO, Glostrup, Denmark; diluted 1:500, incubated for 16 h); NeuN (Chemicon, Temecula, CA; diluted 1:2,000, incubated for 16 h); GAD67 (Chemicon; diluted 1:2,000, incubated for 16 h); calbindin (Chemicon; diluted 1:2,000, incubated for 16 h); calretinin (Chemicon; diluted 1:5,000, incubated for 16 h); and MAP2 (Chemicon; diluted 1:1,000, incubated for 16 h). For staining we used the Vectastain Elite ABC kits (Vector Labs, Burlingame, CA) and diaminobenzidine (Vector Labs). Nissl staining was performed according to standard protocols. Timm staining was performed as described previously (5). Sections were photographed using a Leica DMLB microscope and a Leica DC300F camera (Leica, Bensheim, Germany). The numbers of *c-fos*- or zif-268-positive cells were counted manually or with a CellExplorer 2003 program (BioSciTec, Germany) by investigators blinded to genotype and treatment. For each group of mice, two sections from each of four animals were analyzed.

Endocannabinoid levels. Mice (six to eight per group) were sacrificed 20 min after injection of 20 mg/kg kainate, and hippocampi were rapidly dissected, snap-frozen, and stored at -80°C. Endocannabinoids were extracted, and their

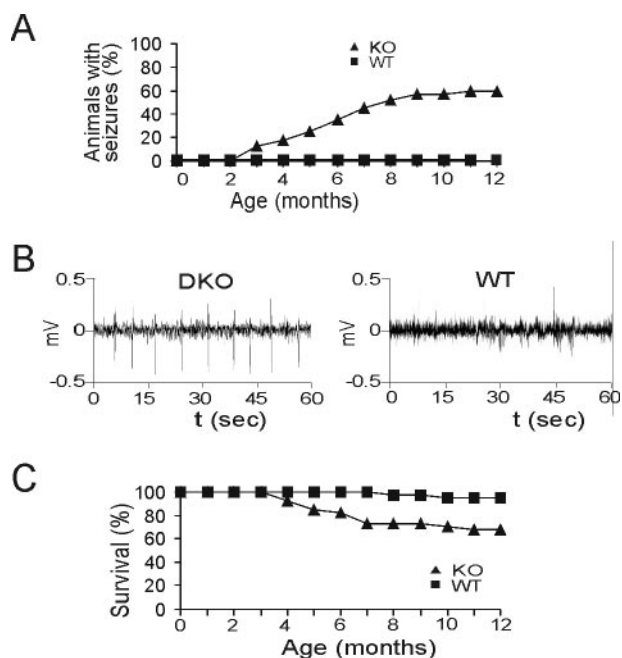


FIG. 1. Spontaneous epileptic seizures in fb-G_{α_q}/G_{α₁₁}-DKO mice. (A) Percentages of fb-G_{α_q}/G_{α₁₁}-DKO mice (KO; *n* = 30) (triangles) and control mice (WT; *n* = 30) (squares) with epileptic seizures. (B) Cortical EEGs from an fb-G_{α_q}/G_{α₁₁}-DKO mouse during a myoclonic seizure (DKO) and from a wild-type mouse without seizures (WT). (C) Percentages of postnatal survival of fb-G_{α_q}/G_{α₁₁}-DKO mice (KO; *n* = 30) (triangles) and control mice (WT; *n* = 30) (squares).

levels were measured by isotope dilution liquid chromatography-mass spectrometry as described previously (40).

Electrophysiology. Hippocampal sections from 3- to 5-week-old forebrain-specific G_{α_q}/G_{α₁₁} double knockout mice and wild-type mice were prepared as described previously (53). Recordings were made from CA1 pyramidal neurons in slices with patch pipettes pulled from 1.5-mm-outer-diameter, thick-walled glass tubing (1511-M; Friedrich & Dimmock). Whole-cell tight seals (>2 GΩ) were made on the soma under visual control with a 40× water immersion lens. Cells were identified as pyramidal neurons by using both electrical and anatomical criteria. Capacitance was fully compensated for by a patch clamp amplifier (EPC-9; HEKA). The holding potential was -60 or -70 mV. The range of series resistances we accepted for recordings was 10 to 15 MΩ. Bipolar stimulation electrodes constructed from thin tungsten wires (50-μm outer diameter) were placed on the stratum radiatum in the CA2 to CA3 pyramidal regions. For inhibitory postsynaptic current (IPSC) recording, the pipette solution contained 130 mM CsCl, 5 mM KCl, 5 mM NaCl, 10 mM HEPES, 0.6 mM EGTA, 4 mM Mg-ATP, and 0.3 mM GTP, with the pH adjusted to 7.3 with NaOH. A non-*N*-methyl-D-aspartate (non-NMDA) receptor antagonist (6-cyano-7-nitroquinoxaline-2,3-dione, 20 μM) and an NMDA antagonist (DL-2-amino-5-phosphonovaleic acid, 50 μM) were always added to the perfusing solution. The CB₁ receptor agonist WIN55,212-2 {*R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-1-(1-naphthalenyl)methanone mesylate} was applied at 5 mM. Single traces are averaged from five consecutive IPSCs acquired before and at the end of the WIN55,212-2 application. Chemicals were obtained from Sigma-Aldrich Co.

Statistics. All data except electrophysiological data are presented as means ± standard error of the means; electrophysiological data are presented as means ± standard deviations. Data were analyzed by analysis of variance followed by Bonferroni's post-hoc test. Statistical analysis was performed by repeated-measure two-way analysis of variance for the factors "treatment" and "genotype" and the interaction "treatment/genotype."

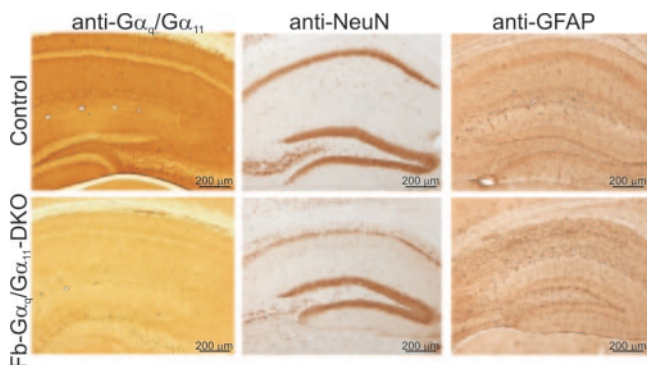


FIG. 2. Histological changes in hippocampi of fb- $G\alpha_q/G\alpha_{11}$ -DKO mice. Hippocampal sections from 8-month-old control mice (upper row) and fb- $G\alpha_q/G\alpha_{11}$ -DKO mice (lower row) stained with antibodies against $G\alpha_q/G\alpha_{11}$, NeuN, and GFAP proteins.

RESULTS

Starting at the age of about 3 months, fb- $G\alpha_q/G\alpha_{11}$ -DKO mice showed spontaneous epileptic seizures, and both the frequency of seizures in each animal and the number of affected animals increased with age (Fig. 1A). The most frequent forms of seizure were mild myoclonic seizures, and combined EEG and video monitoring of mutant mice showed a series of rhythmic spikes (0.2 ± 0.03 Hz; 45 s, 7-min duration; 0 to 5 times a day) (Fig. 1B, left panel) concomitant with myocloni of the body. Such episodes were not observed in wild-type animals (Fig. 1B, right panel). Some animals also experienced clonic-tonic seizures, leading to an age-dependent reduction of survival (Fig. 1C).

Histological and immunohistochemical analyses of mice younger than 3 months did not show abnormalities (data not shown). However, older mice, especially those with symptomatic epilepsy, showed neuronal degeneration and reactive gliosis in the hippocampal CA1 region, indicative of temporal lobe epilepsy (Fig. 2). In contrast to other models of temporal lobe epilepsy, we did not observe mossy fibers sprouting in sections visualized by Timm staining (data not shown).

To investigate whether abnormalities in neuronal excitability are present before the onset of spontaneous seizures, we studied the effects of chemical convulsants in 6- to 10-week-old fb- $G\alpha_q/G\alpha_{11}$ -DKO mice *in vivo*. We found that the application of the glutamate receptor agonist KA or the gamma aminobutyric acid type A ($GABA_A$) receptor antagonist PTZ induced stronger seizures in fb- $G\alpha_q/G\alpha_{11}$ -DKO mice than in wild-type mice (Fig. 3A and C) and that mutant mice exhibited seizures at dosages that were already subconvulsive in wild-type mice (Fig. 3A). Latency to seizure onset was decreased in fb- $G\alpha_q/G\alpha_{11}$ -DKO mice (Fig. 3B and D), while seizure-associated lethality was strongly increased (data not shown). Mice with a single deficiency in $G\alpha_q$ or $G\alpha_{11}$ showed mildly increased seizure susceptibility when challenged with kainic acid (see Fig. S1 in the supplemental material).

KA-induced seizures have been shown to be accompanied by enhanced expression of immediate early genes like *c-fos*, *fosB*, or *zif-268* in the hippocampi of wild-type mice (30), and especially *c-fos* expression appears to be critically involved in the protection against KA-induced excitotoxicity (40, 70). To test

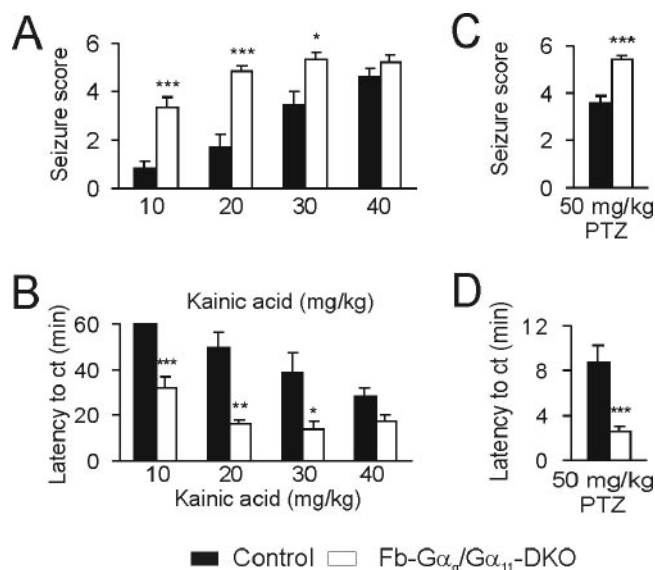


FIG. 3. Increased sensitivities toward chemical convulsants of 6- to 10-week-old fb- $G\alpha_q/G\alpha_{11}$ -DKO mice compared with those of control mice. Seizure scores (A and C) and latency periods (B and D) to first clonic-tonic (ct) convulsions after intraperitoneal application of kainic acid (A and B) or PTZ (C and D) in control and fb- $G\alpha_q/G\alpha_{11}$ -DKO mice (five to six mice per group). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Seizures were scored as described previously (28).

whether the impaired activation of potentially neuroprotective pathways may contribute to increased seizure susceptibility in fb- $G\alpha_q/G\alpha_{11}$ -DKO mice, we investigated the expression of *c-fos* and *zif-268* proteins in untreated and KA-treated wild-type and mutant mice (Fig. 4). Compared to the upregulation in wild-type mice, the KA-induced upregulation of both proteins was almost abrogated in hippocampi from fb- $G\alpha_q/G\alpha_{11}$ -DKO mice (Fig. 4), suggesting that neuroprotective mechanisms are impaired in these mice.

Since increased excitability and impaired immediate early gene activation are also observed in mice lacking endocannabinoid CB_1 receptors in principal forebrain neurons and since the G_q/G_{11} G proteins have been involved in endocannabinoid synthesis, we determined the levels of anandamide and 2-AG in the hippocampi of wild-type and fb- $G\alpha_q/G\alpha_{11}$ -deficient mice (Fig. 5). Under basal conditions, anandamide levels were significantly lower in mutant mice than in controls (Fig. 5A), while 2-AG levels did not differ significantly (Fig. 5B). The chemical convulsant KA enhanced both anandamide and 2-AG levels in control mice (Fig. 5A and B). Interestingly, KA treatment still increased anandamide levels in fb- $G\alpha_q/G\alpha_{11}$ -DKO mice but failed to induce 2-AG synthesis (Fig. 5A and B). In mice with a single deficiency in $G\alpha_q$ or $G\alpha_{11}$, KA-induced synthesis of 2-AG was also impaired, but the reduction was less prominent than in fb- $G\alpha_q/G\alpha_{11}$ -deficient mice (see Fig. S1 in the supplemental material). These data indicate that basal hippocampal levels of anandamide, but not of 2-AG, are under tonic control of the G_q/G_{11} -mediated signaling pathway. In contrast, G_q/G_{11} -mediated signaling is necessary for KA-induced formation of 2-AG but not of anandamide.

To further test whether the reduced formation of endocan-

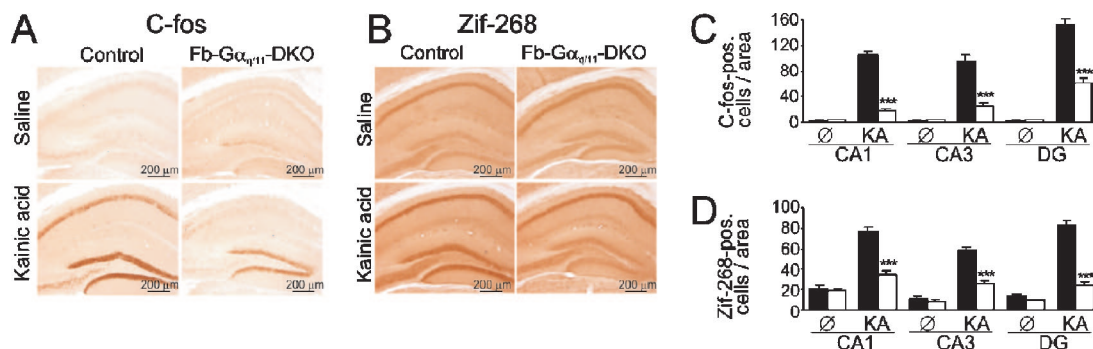


FIG. 4. Kainate-induced upregulation of immediate early genes is impaired in fb-Gα_q/Gα₁₁-DKO mice. (A and B) Staining with antibodies against *c-fos* (A) and *zif-268* (B) proteins in the hippocampi of control and fb-Gα_q/Gα₁₁-DKO mice 45 min after intraperitoneal injection of saline or 20 mg/kg KA. (C and D) Statistical evaluation of the number of *c-fos*-positive (C) or *zif-268*-positive (D) cells in hippocampal regions CA1 and CA3 and the dentate gyrus (DG) (four mice per group). Black bars, control mice; white bars, fb-Gα_q/Gα₁₁-DKO mice; ***, *P* < 0.0001 versus kainate wild-type mice.

nabinoids in the absence of Gα_q/Gα₁₁ is involved in abnormal neuronal excitability and impaired neuroprotection, we pre-treated mice with the CB₁ receptor antagonist SR141716A. As expected, SR141716A strongly aggravated KA-induced seizures in wild-type mice (Fig. 6A). However, in fb-Gα_q/Gα₁₁-DKO mice, a blockade of CB₁ receptors did not further aggravate KA-induced seizures (Fig. 6A). In this experiment, KA was administered at the reduced dosage of 10 mg/kg to detect the potential aggravation of seizure intensity in fb-Gα_q/Gα₁₁-DKO mice as well, which have per se higher seizure scores than wild-type mice (see Fig. 3). Since SR141716A is a competitive antagonist at the CB₁ endocannabinoid receptor, the lack of SR141716A aggravation of KA-induced seizures in fb-Gα_q/Gα₁₁-DKO mice supports our finding that endogenous levels of endocannabinoids are significantly decreased in fb-Gα_q/Gα₁₁-DKO mice, at least at those synapses involved in CB₁-mediated neuroprotection. To enhance endocannabinoid levels selectively at sites of physiological production, we treated mice with the endocannabinoid reuptake inhibitor OMDM-2 (54). OMDM-2 caused a slight but nonsignificant amelioration of seizures induced by 30

mg/kg KA in wild-type mice but a strong amelioration in fb-Gα_q/Gα₁₁-DKO mice (Fig. 6B), suggesting that endocannabinoid-mediated effects are basically functional in mutant mice. To further substantiate this notion, we investigated cannabinoid-mediated retrograde inhibition of synaptic transmission by electrophysiological means. In both the

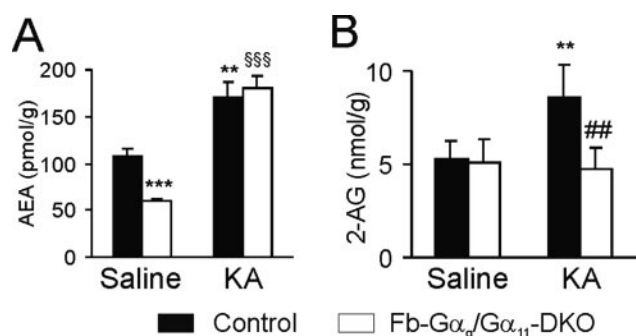


FIG. 5. Endocannabinoid levels in hippocampi of fb-Gα_q/Gα₁₁-DKO mice. (A and B) Anandamide (AEA) (A) and 2-AG (B) levels were determined by isotope dilution liquid chromatography-mass spectrometry in hippocampi from control and fb-Gα_q/Gα₁₁-DKO mice after intraperitoneal application of saline or 20 mg/kg KA (six to eight mice per group). **, *P* < 0.01; ***, *P* < 0.001 versus saline in wild-type mice; \$\$\$, *P* < 0.001 versus saline in fb-Gα_q/Gα₁₁-DKO mice; ##, *P* < 0.01 versus KA in wild-type mice.

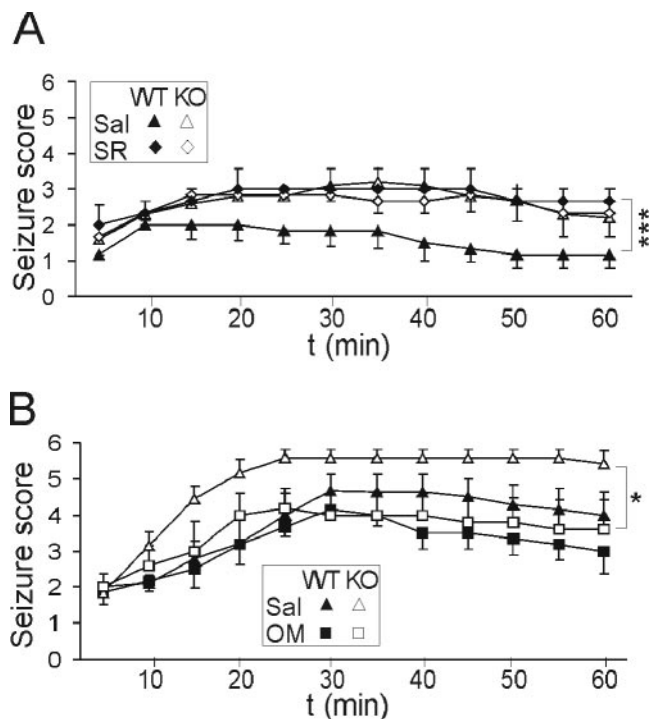


FIG. 6. Pharmacological manipulation of the endocannabinoid system in wild-type and fb-Gα_q/Gα₁₁-DKO mice. (A and B) Effect of the competitive CB₁ antagonist SR141716A (A) and the endocannabinoid reuptake inhibitor OMDM-2 (B) on kainic acid-induced seizures. The fb-Gα_q/Gα₁₁-DKO (KO) and control (WT) mice received saline (Sal), 3 mg/kg SR141716A (SR), or 5 mg/kg OMDM-2 (OM) intraperitoneally 30 min before the application of kainic acid (at 10 [A] or 30 [B] mg/kg KA; six to eight mice per group). *, *P* < 0.05, saline-treated versus OMDM-2-treated fb-Gα_q/Gα₁₁-DKO mice; ***, *P* < 0.001, saline-treated versus SR-treated wild-type mice.

wild-type and the mutant hippocampal slices, the application of the CB₁ receptor agonist WIN55,212-2 caused a significant reduction in the amplitude of IPSCs in CA1 hippocampal neurons (see Fig. S2 in the supplemental material). Taken together, both pharmacological and electrophysiological data indicate that endocannabinoid-mediated protective mechanisms are basically intact in fb-G α_q /G α_{11} -DKO mice but that endogenous cannabinoid production is impaired.

DISCUSSION

In this study we have shown that genetic inactivation of the G $_q$ /G $_{11}$ -mediated signaling pathway in principal forebrain neurons results in increased neuronal excitability. This finding was unexpected since the modulatory effects of G $_q$ /G $_{11}$ -mediated signaling are believed to facilitate rather than dampen neuronal activity. However, increased seizure susceptibility was also found in mice lacking PLC- β 1 (31), the inositol 1,4,5-trisphosphate receptor (42), or the G $_q$ /G $_{11}$ -coupled 5-HT_{2c} receptor (60), suggesting that the G $_q$ /G $_{11}$ -mediated signaling pathway indeed exerts important inhibitory effects.

Only a few G $_q$ /G $_{11}$ -mediated effects that might promote neuronal inhibition have been described. G $_q$ /G $_{11}$ -coupled receptors are present on hippocampal interneurons (26, 64), and impaired activation of these cells might shift the balance between excitation and inhibition. However, since Cre-mediated recombination in the *Camkcre4* mouse line is restricted to principal neurons (40), this model obviously does not apply here. Some G $_q$ /G $_{11}$ -coupled receptors, e.g., the M₁ acetylcholine receptor, have been suggested to negatively regulate N-type calcium channels through a breakdown of membrane phosphatidylinositol 4,5-bisphosphate (29) and thereby might contribute to inhibition. However, given the fact that a majority of inhibitory presynaptic G protein-coupled receptors, such as adenosine A₁, α_2 adrenergic μ and δ opioid, and GABA_B receptors, signal through G $_{i/o}$ family G proteins (17, 45), the contribution of G $_q$ /G $_{11}$ -coupled receptors to calcium channel regulation seems to be rather small. Also, the endocannabinoid CB₁ receptor is known to mediate its presynaptic inhibitory effects through G $_{i/o}$ family G proteins (27, 28, 47, 55). It was recently reported that the synthetic CB₁ agonist WIN55,212-2 induces a calcium response in CB₁-transfected HEK293 cells in a G $_q$ /G $_{11}$ -dependent manner; however, natural CB₁ agonists such as Δ^9 -tetrahydrocannabinol, 2-AG, and anandamide analogues did not elicit such responses (36).

Though not directly coupled to CB₁, the G $_q$ /G $_{11}$ -mediated signaling pathway was suggested to contribute to endocannabinoid functions through its role in endocannabinoid synthesis. We found that hippocampal levels of anandamide, but not of 2-AG, are reduced in the absence of G α_q /G α_{11} , indicating that anandamide formation is under the tonic control of receptors coupled to these G proteins, while 2-AG formation is not. Muscarinic and purinergic receptors are among the G protein-coupled receptors that might contribute to basal anandamide levels as they are able to induce anandamide formation in the absence of extracellular Ca²⁺ as well (61). On the other hand, in the formation of basal 2-AG levels as previously described,

PLC- β -independent biosynthetic routes (4, 59) seem to predominate.

In contrast to basal 2-AG production, G $_q$ /G $_{11}$ -mediated signaling is indispensable for hippocampal 2-AG formation following KA application, and this might be due to the fact that DAG, a fundamental precursor of that pool of 2-AG that acts as an endocannabinoid (13, 15, 70), is mostly formed in depolarized neurons following the activation of PLC- β (58). In contrast to 2-AG formation in response to KA, G $_q$ and G $_{11}$ are not necessary for KA-induced anandamide formation. This observation supports the concept that anandamide can be produced in response to either metabotropic (61) or ionotropic (15) receptor stimulation alone, provided that the intracellular Ca²⁺ concentration is increased in order to induce the activation of Ca²⁺-sensitive biosynthesizing enzymes and the subsequent processing of non-PLC- β -derived biosynthetic precursors (54). With respect to the differential role of the two endocannabinoids in control of excitability, it is important to note that in wild-type mice, KA-induced 2-AG concentrations are at least 50-fold higher than the anandamide concentrations, and the loss of KA stimulation therefore results in much lower total endocannabinoid concentrations in fb-G α_q /G α_{11} -DKO mice than in wild-type mice (a net increase of 0.06 plus 3.35 or 3.41 nmol/g tissue in wild-type mice compared to a net decrease of 0.12 to 0.3 or -0.18 nmol/g tissue in fb-G α_q /G α_{11} -DKO mice) (Fig. 5).

The phenotype of CB₁ receptor-deficient mice resembles that of fb-G α_q /G α_{11} -DKO mice in that both mouse strains have increased seizure susceptibilities in response to KA and inability to induce expression of immediate early genes encoding the *c-fos* and *zif-268* transcription factors, which are believed to counteract excitotoxic damage (40, 70). This supports the hypothesis that impaired endocannabinoid-mediated retrograde signaling underlies increased neuronal excitability in fb-G α_q /G α_{11} -DKO mice. However, forebrain-specific CB₁-deficient mice seem to be less prone to spontaneous epilepsy, although seizures have been observed in these animals, especially during the stress of handling (B. Lutz, personal communication). This phenotypic difference might be explained by the presence of non-CB₁ (13, 16, 19, 56) receptors on hippocampal principal neurons, which contributes to endocannabinoid-mediated neuroprotection. In addition, defects in other G $_q$ /G $_{11}$ -mediated processes which occur independently of endocannabinoids and which under normal conditions decrease neuronal activity, like the above-mentioned negative regulation of N-type Ca²⁺ channels, may contribute to the more-severe phenotype observed in fb-G α_q /G α_{11} -DKO mice.

Taken together, our data suggest that the G $_q$ /G $_{11}$ -mediated signaling pathway is critically involved in endocannabinoid synthesis under both basal and excitotoxic conditions. The phenotypic similarities between forebrain-specific CB₁-deficient mice and pharmacological *in vivo* data indicate that impaired endocannabinoid formation is associated with increased seizure susceptibility and impaired neuroprotection. However, defective endocannabinoid synthesis may not account exclusively for the increased neuronal excitation in the absence of G $_q$ /G $_{11}$, and other G $_q$ /G $_{11}$ -mediated inhibitory mechanisms may contribute to the phenotype.

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