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KCNE2 forms potassium channels with KCNA3 and KCNQ1 in the choroid plexus epithelium


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ABSTRACT Cerebrospinal fluid (CSF) is crucial for normal function and mechanical protection of the CNS. The choroid plexus epithelium (CPe) is primarily responsible for secreting CSF and regulating its composition by mechanisms currently not fully understood. Previously, the heteromeric KCNQ1-KCNE2 K+ channel was functionally linked to epithelial processes including gastric acid secretion and thyroid hormone biosynthesis. Here, using including gastric acid secretion and thyroid hormone biosynthesis. Here, using

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Cerebrospinal fluid (CSF), secreted by the ependymal cells of the choroid plexus (CP), has numerous important functions, including physical support for and protection of the CNS, delivery of nutrients to and removal of waste products from the CNS, and physical communication between different CNS structures. Abnormal CSF production and/or absorption can cause common clinical problems, including cerebral edema and intracranial hypertension; drugs that reduce CSF production are effective for treating these conditions (1). Identification of novel drug targets in the choroid plexus epithelium (CPe) to manipulate specific CPe transport processes could open therapeutic avenues for a diverse range of disorders (2).

Voltage-gated potassium (Kv) channel α subunits KCNAA1, KCNA3, and KCNA6 (also named Kv1.1, Kv1.3, and Kv1.6, respectively) and inward rectifier K+ channel α subunit KCNJ13 (Kir7.1) have all been detected at the CPe apical membrane. They are thought to contribute to apical K+ efflux (3–5), potentially providing a leak pathway for K+ ions pumped into the cell by the Na+-K+ -ATPase and to maintenance of the negative cell membrane potential (Em), which is important in establishing the electrochemical gradient for anion efflux needed to drive CSF secretion (3). CPe K+ transport from the CSF to the blood is vital in maintaining CSF [K+] close to 3 mM, even when plasma [K+] is experimentally varied over a range of 2 to 11 mM (6) and is thus essential for the normal activity of the CNS.

Kvα subunits form complexes with a range of ancillary subunits, such as the single-transmembrane domain proteins encoded by the KCNE gene family (ref. 7 and Fig. 1A). We previously found that variants in the gene encoding KCNE2 (a protein we originally termed MinK-related peptide 1) associate with inherited and drug-induced forms of the cardiac arrhythmia, long QT syndrome (LQTS; refs. 8, 9). More recently, KCNE2 sequence variants or gene duplications have been tentatively associated with a human cardiocerebral phenotype comprising both neonatal seizures and LQTS (10) and with schizophrenia (11), suggesting potential cerebral functions for KCNE2, but KCNE2 transcript levels in human neuronal tissue appear relatively low (12). However, KCNE2 transcripts are reportedly highly enriched in the fourth and lateral ventricles.
Frozen samples were cut into 10-μm-thick sections (Cryo-tek, Leica Microsystems, Bannockburn, IL, USA). Immunohistochemical detection of Kcnq1 and Kcne2 was performed with a Discovery XT processor (Ventana Medical Systems, Tucson, AZ, USA). Goat polyclonal anti-KCNQ1 (pan-species) primary antibody (SC-10646; Santa Cruz Biotechnology, Inc.) was used at 1 mg/ml, in-house rabbit polyclonal, site-directed anti-KCNE2 (pan-species) serum was diluted 1:5000 after column-enriching IgG, mouse monoclonal anti-KCNA3 (75-009; NeuroMab, University of California, Davis, CA, USA) was diluted 1:500, and rabbit or goat polyclonal anti-NKCC1 (Santa Cruz Biotechnology, Inc.) was diluted 1:1000. Preceding the primary antibody incubation, tissue sections were blocked for 30 min in 10% normal goat serum (for slides stained with a combination of mouse and rabbit primary and secondary antibodies), rabbit serum (for slides stained with a combination of mouse and goat primary and secondary antibodies), or mouse serum (for slides stained with a combination of rabbit and goat primary and secondary antibodies) and 2% BSA in PBS, followed by an 8-min avidin/biotin block. The primary antibody incubation (3 h) was followed by incubation with biotinylated anti-rabbit, anti-mouse, or anti-goat antibodies at 1:200 dilution (Vector Laboratories, Burlingame, CA, USA). Immunofluorescence detection of Kcne2, Kcnq1, Kcna3, and Nkcc1 was performed using a Discovery XT processor (Ventana Medical Systems), Goat polyclonal anti-KCNQ1 (pan-species) primary antibody (SC-10646; Santa Cruz Biotechnology, Inc.) was used at 1 mg/ml, in-house rabbit polyclonal, site-directed anti-KCNE2 (pan-species) serum was diluted 1:5000 after column-enriching IgG, mouse monoclonal anti-KCNA3 (75-009; NeuroMab, University of California, Davis, CA, USA) was diluted 1:500, and rabbit or goat polyclonal anti-NKCC1 (Santa Cruz Biotechnology, Inc.) was diluted 1:1000. Preceding the primary antibody incubation, tissue sections were blocked for 30 min in 10% normal goat serum (for slides stained with a combination of mouse and rabbit primary and secondary antibodies), rabbit serum (for slides stained with a combination of mouse and goat primary and secondary antibodies), or mouse serum (for slides stained with a combination of rabbit and goat primary and secondary antibodies) and 2% BSA in PBS, followed by an 8-min avidin/biotin block. The primary antibody incubation (3 h) was followed by incubation with biotinylated anti-rabbit, anti-mouse, or anti-goat IgG as appropriate (ABC kit; Vector Laboratories). The secondary detection was performed with streptavidin-horseradish peroxidase D (Ventana Medical Systems), followed by incubation with tyramide Alexa Fluor 488 (Invitrogen, Carlsbad, CA, USA) or tyramide Alexa Fluor 568 (Invitrogen). Immunostained slides were viewed with a Zeiss Axiovert 200 widefield microscope, and pictures were acquired using MetaMorph 7.1 software (Molecular Devices, Sunnyvale, CA, USA).

Choroid plexus harvesting and preparation for cellular electrophysiology or biochemistry

Adult Kcne2<sup>+/+</sup> and Kcne2<sup>−/−</sup> mice were killed by CO<sub>2</sub> asphyxiation, and the choroid plexus was removed from the fourth ventricle of the brain. Tissue samples were then kept in ice-cold, control bath solution (140 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM HEPES, 4 mM mannitol, and 5 mM glucose, adjusted to pH 7.3 with NaOH; osmolality=298±4 mosmol/kg H<sub>2</sub>O, n=6) and used in patch-clamp experiments within 3 h of isolation; or snap-frozen in liquid N<sub>2</sub> for use in Western blot analysis.

CSF extraction and ion quantification

CSF was isolated from the cisterna magna of 3- to 6-month-old Kcne2<sup>+/+</sup> and Kcne2<sup>−/−</sup> mice using a modification of a technique described previously (17). In brief, mice were initially anesthetized with 1% isoflurane in 100% oxygen in a Plexiglas chamber and then placed in a small animal stereotactic apparatus (model 900: David Kopf Instruments, Tujunga, CA, USA) fitted with a mouse anesthesia mask (David Kopf Instruments) and ear bars to minimize movement of the head. With the head locked in place, the body of the mouse was allowed to drop to give access to the neck region. The neck was shaved, an incision was made using a sterile scalpel (no. 10 blade), and the muscles...
of the neck were separated by blunt dissection to reveal the cisterna magna. Glass borosilicate capillary tubes (B100–78-10; Sutter Instrument, Novato, CA, USA), pulled on a vertical micropipette puller (PP-830; Narishige, Tokyo, Japan) to generate a tapered tip with an inner diameter of roughly 0.6 mm, were then used to penetrate the cisterna magna, and CSF was drawn into the capillary tube. A 1-mL syringe with polyethylene tubing attached to the end (to allow tight connection with the capillary tube) was then used to remove the CSF from the capillary tube. CSF samples were placed on dry ice and stored at −80°C until analysis. Mice were then killed by CO₂ asphyxiation.

CSF [K⁺] was analyzed by AniLytics (Gaithersburg, MD, USA) using a K⁺-ion-selective electrode. CSF pH measurements were conducted using a pH glass microelectrode (Microelectrodes, Inc., Bedford, NH, USA). CSF [Cl⁻] was determined using a QuantiChrom colorimetric chloride assay kit (Bioassay Systems, Hayward, CA, USA) following the manufacturer’s protocol. In brief, nonpooled 1-μL CSF samples from 6-mo-old Kcn2⁺/⁺ and Kcn2⁻/⁻ mice were diluted 20-fold, as per the manufacturer’s protocol for biological samples, and 5 μL of each dilution was independently tested 6 times for [Cl⁻]. The absorbance of each data point was read at 610 nm using a VERSAmax tunable microplate reader (Molecular Devices) and analyzed using SoftMax Pro 4.8 (Molecular Devices). The absorbance readings were converted to [Cl⁻] (mM) by direct comparison to a [Cl⁻] standard curve.

Western blot analysis

Cell lysates from choroid plexus epithelia of the fourth ventricle were pooled from either 20 Kcn2⁺/⁺ or Kcn2⁻/⁻ mice and isolated according to Speake et al. (18) with centrifugation to remove nuclear material, or whole CPe lysates from individual mice without removal of nuclear material were used. This difference in preparation had no noticeable effect on membrane protein subunit band patterns, but if nuclear material was not removed, an additional glyceraldehyde 3-phosphate dehydrogenase (GAPDH) band was observed. The lysates were resolved using SDS-PAGE and transferred to PVDF membranes for Western blot analysis using standard techniques and equipment (Bio-Rad Laboratories, Hercules, CA, USA). PVDF membranes were incubated for 3 h at room temperature in 5% milk with primary antibodies, diluted as follows: rabbit monoclonal Kcn3 (NeuroMab) 1:500; mouse monoclonal Kcn1 (Chemicon International, Temecula, CA) 1:1000; mouse monoclonal Kcn6 (Chemicon International) 1:500; goat polyclonal Kcnj13 (Santa Cruz Biotechnology, Inc.) 1:1000; mouse monoclonal Kcne1 (Santa Cruz Biotechnology, Inc.) 1:1000; mouse monoclonal Kcne2 (NeuroMab) 1:500; mouse monoclonal Kcne1 (Chemicon International, Temecula, CA) 1:1000; mouse monoclonal Kcne6 (Chemicon International) 1:500; goat polyclonal Kcnj13 (Santa Cruz Biotechnology, Inc.) 1:1000; NKCC1 (Santa Cruz Biotechnology, Inc.) 1:500; AQPI (Chemicon International) 1:1000; and GAPDH (Bio-Rad Laboratories) 1:100. For secondary detection, horseradish peroxidase-conjugated species-appropriate anti-IgG antibodies (1:10,000, Bio-Rad Laboratories) were used. Signals were detected with ECL-Plus chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA), were visualized by fluorography, and, where indicated, were quantified using densitometry as we reported previously (16).

Electrophysiology

K⁺ channel activity in CPe cells isolated from 3-mo-old Kcn2⁺/⁺ and Kcn2⁻/⁻ mice was recorded by whole-cell patch-clamp methods. Briefly, small pieces of choroid plexus tissue, bathed in the control bath solution, were affixed to Cell-Tak-coated coverslips and transferred to a perfusion chamber (bath volume 400 μL) mounted on the stage of an inverted microscope (Olympus, Tokyo, Japan). Patch pipettes were made from hematocrit capillary tubes (Oxford Labware, Mansfield, MA, USA) with a vertical micropipette puller. The tip resistances of the patch pipettes were 3–5 MΩ. Conventional whole-cell patch-clamp recordings were performed at 22–25°C after gigahm seals had been obtained on the exposed apical membrane of the epithelial cells. Whole-cell currents (voltage-clamp mode)
plexus lysate from individual mice (as in the blot shown here; hence, 2 bands are observed for GAPDH, corresponding to nuclear and cytosolic forms of GAPDH). B, C) Western blots of mouse Kcnq1 and Kcne2-/- and Kcne2+/+ CPe cell lysates, using commercial antibodies raised against the various ion channel α subunits (B) and transporters (C) shown and GAPDH as a loading control. Representative of n = 3 experiments, each using either pooled choroid plexus lysates with nuclear fraction removed by centrifugation from 20 mice/genotype or whole choroid plexus lysate from individual mice. D) Mean fold change in band density for Kcnq1 (n=3) and Kcne2 protein (n=4) in Kcne2+/+ vs. Kcne2-/- CPe cell lysates (i.e., effect of Kcne2 deletion). Error bars = SE.

RESULTS

Kcne2 and Kcnq1 are expressed in the CPe

Western blotting of mouse CPe lysates, with Kcne2-/- mouse CPe lysates as a negative control, indicated Kcne2 expression in the Kcne2+/+ mouse CPe (Fig. 1B). Furthermore, using immunohistochemical analysis and with Kcne2-/- mouse CPe lysates or omission of primary antibody as negative controls, we found cerebral Kcne2 protein expression to be strikingly enriched in the apical membrane of the CPe of both embryonic (Fig. 1C) and adult (Fig. 1D) mice.
Figure 4. Kcne2 regulates Kcnq1 and Kcna3, but not Kcnal, in the CPe. A–D Top panels: exemplar whole-cell native CPe currents sensitive to the agents indicated [A] 10 μM XE991; B] 10 μM margatoxin (MgTX); [C] 100 nM dendrotoxin (DTX)], computed using digital subtraction of postdrug currents from predrug currents and the voltage protocol indicated. Dashed line indicates 0 current level. Bottom panels: mean current density-voltage relationship for drug-sensitive currents as above; n = 7 cells/genotype for Kcne2+/+ and Kcne2−/− (XE991); n = 4 cells/genotype for Kcne2+/− and n = 7 cells/genotype for Kcne2+/− (MgTX); and n = 5 cells/genotype for Kcne2+/− and n = 6 cells/genotype for Kcne2+/+ (DTX). *P < 0.05.

We also discovered that Kcnq1, a K+ channel α subunit partner of Kcne2 in the stomach and thyroid (15, 19), is expressed in the CPe. Using Western blotting of lysates pooled from the CPe of 20 mice and GAPDH expression as a loading control, we discovered that Kcne2 deletion resulted in up-regulation of CPe Kcnq1 expression (Fig. 2A, D), as we previously observed in the stomach mucosa of Kcne2−/− mice (15). Analyzing the other known CPe K+ channels, again using Western blotting of CPe lysates pooled from 20 mice/genotype, we found that Kcne2 deletion appeared to decrease CPe Kcna3 protein expression but had no effect on the expression of the other known CPe K+ channel proteins, Kcna1 (Kv1.1), Kcna6 (Kv1.6), or Kcnj15 (Kir7.1) (Fig. 2B, D). Similarly, Kcne2 deletion did not alter levels of two other known CPe membrane proteins, aquaporin 1 (AQP1) and NKCC1 (Fig. 2C).

Kcne2 deletion increases CPe cell Kcna3 and Kcnq1 outward current density

Patch-clamp recordings in whole-cell configuration from isolated Kcne2+/+ mouse CPe cells bathed in 5 mM K+ solution exhibited an almost linear current-voltage relationship, whereas Kcne2−/− CPe cell K+ current exhibited increased outward rectification, with a 2-fold increased mean outward current density compared with Kcne2+/+ CPe cells (Fig. 3A, B). The outward current in Kcne2−/− CPe cells also exhibited slower voltage-dependent activation and faster inactivation than that of Kcne2+/+ CPe cells (Fig. 3C).

We next used Kv channel antagonists to determine the molecular correlates of the outward current altered by Kcne2 deletion, focusing on Kcna1, Kcna3, and Kcnq1. The mean density of the CPe outward current component sensitive to the Kcnq-specific antagonist XE991 was increased 25% by Kcne2 deletion, and the outward rectification of the XE991-sensitive current was also increased (Fig. 4A). CPe outward current sensitive to the Kcna3-specific antagonist margatoxin was increased 2-fold by Kcne2 deletion, with the small amount of inward current remaining unchanged (Fig. 4B). In contrast, CPe Kcna1 current, identified by dendrotoxin sensitivity, was unchanged by Kcne2 deletion (Fig. 4C).

These changes in XE991-sensitive current (Fig. 4A) are consistent with loss of Kcne2 from Kcnq1-Kcne2 complexes, because Kcne2 reduces the voltage dependence of Kcnq1 activation (and thus reduces outward rectification) and decreases its macroscopic outward current amplitude (20). The changes could also reflect the increase in Kcnq1 protein membrane expression suggested by data herein (Fig. 2A).

The increased Kcna3 current appeared paradoxical because total Kcna3 protein expression in the CPe was reduced by Kcne2 deletion (Fig. 2B). Furthermore, Kcne2 has not previously been reported to

Figure 5. Kcne2 partially suppresses Kcn3 but not Kcnal current in vitro. A) Exemplar whole-cell currents in CHO cells transfected with rat Kcn3 cDNA alone or with mouse Kcne2 cDNA, using 5 mM K+ bath solution and the protocol indicated. Dashed line indicates 0 current level. B) Mean current density-voltage relationship for cells as in A; n = 8–9 cells/group. *P < 0.001 for outward currents. C) Exemplar whole-cell currents in CHO cells transfected with rat Kcnal cDNA alone or with mouse Kcne2 cDNA, using 5 mM K+ bath solution and the protocol indicated. Dashed line indicates 0 current level. D) Mean current density-voltage relationship for cells as in C; n = 15–18 cells/group. P > 0.05 (no significant difference between groups).
modulate Kcn3, and human KCNE2 was previously found to have no effect on rat Kcn3 function when coexpressed in human embryonic kidney (HEK) cells (21). To understand this apparent paradox, we determined the functional effects of murine Kcne2 and Kcn3 heterologous coexpression in CHO cells and found that Kcne2 inhibited Kcn3 current by 60% (in vivo) changes caused by each genotype and thus also for the current density margatoxin-, and dendrotoxin-sensitive) was sufficient of the three current components studied (XE991-, CPe Kcna3 current (Fig. 4B) and also for the lack of effects of Kcne2 deletion on native CPe Kcn1 current (Fig. 4C) and constitutes the first report of Kcn3 modulation by Kcne2 (in vitro or in vitro).

Summation of the CPe current densities for each of the three current components studied (XE991-, margatoxin-, and dendrotoxin-sensitive) was sufficient to account for all of the outward current for each genotype and thus also for the current density changes caused by Kcne2 deletion (Figs. 2 and 3). Thus, Kcne2 deletion increased CPe Kcnq1 and Kcn3 outward current density and outward rectification, with no effect on Kcn1. Furthermore, the functional changes were consistent with direct functional regulation of Kcnq1 and Kcn3 by Kcne2 in wild-type mouse CPe.

**Kcne2 deletion alters the trafficking polarity of Kcn3 and Kcnq1 in the CPe**

Given the functional data suggesting regulation of Kcnq1 and Kcn3 by Kcne2 in the CPe, we examined localization of these subunits in the CPe using immunofluorescence. First, we again detected Kcne2 in the apical membrane of the CPe, using single labeling with anti-Kcne2 antibody, DAPI staining of nuclei, and the CPe of Kcne2−/− mice as a negative control (Fig. 6A). Next, using single labeling with anti-Kcnq1 antibody and DAPI staining of nuclei, we found that Kcnq1 appeared to localize to the apical membrane of the CPe in Kcne2+/+ mice but to the basolateral membrane and/or intracellular compartment of the CPe in Kcne2−/− mice (Fig. 6B).

Using double labeling with rabbit anti-Kcne2 antibody and goat anti-Kcnq1 antibody, we found that Kcne2 and Kcnq1 partially colocalize in the CPe apical membrane. Fainter Kcnq1 staining was also observed in other areas, but where it was strongest at the CPe apical membrane, Kcne2 was strongly colocalized with it (Fig. 6C). The NKCC1 transporter is an established marker of the CPe apical membrane (22); therefore, we next used double-labeling with rabbit anti-NKCC1 antibody and goat anti-Kcnq1 antibody to verify the aberrant Kcnq1 trafficking observed with single labeling in the CPe of Kcne2−/− mice (Fig. 6B). In Kcne2−/− CPe, strongly overlapping Kcnq1 and NKCC1 signals were observed, confined specifically to the CPe (Fig. 6D, E). In contrast, in Kcne2+/+ mice, although anti-NKCC1 antibody staining was consistent with an apical localization as previously reported (22), Kcnq1 staining did not colocalize with NKCC1, but was in the basolateral and/or intracellular compartments (Fig. 6F). Thus, both single- and double-labeling experiments suggested that Kcnq1 colocalized apically with Kcne2 in the CPe of wild-type mice, but that in the CPe of Kcne2−/− mice, Kcnq1 did not reach the apical membrane.

Kcn3 was previously identified in the CPe apical membrane (4). Here, using double labeling with mouse anti-Kcn3 and rabbit anti-Kcne2 antibodies, we found that Kcn3 colocalized with Kcne2 in the Kcne2+/+ CPe, as expected, because both are apical (Fig. 6G). We could not obtain sufficient resolution with this combination of antibodies in wild-type CPe to interpret further than this, although in further support of an apical localization for Kcn3 in Kcne2+/+ CPe, we also observed at least partial colocalization of Kcn3 and NKCC1 in Kcne2+/+ CPe (Fig. 6H). In contrast, when double-labeling CPe from Kcne2−/− mice with rabbit anti-NKCC1 and mouse anti-Kcn3 antibodies, we saw no colocalization: NKCC1 was apically located, but Kcn3 was very distinctly localized to the basolateral compartment (Fig. 6I).

**Kcne2 deletion hyperpolarizes CPe E_m and reduces CSF but not serum [Cl−]**

Given that Kcne2 altered CPe K+ currents, which are frequently influential in setting resting E_m, we next measured resting E_m in CPe cells isolated from Kcne2+/+ and Kcne2−/− mice. These experiments revealed a statistically significant 9 ± 2 mV hyperpolarizing shift due to Kcne2 deletion (Table 1). This result is consistent with the increased K+ efflux we observed in CPe cells isolated from Kcne2−/− mice (Figs. 3 and 4), as K+ conductance drives the cell membrane potential toward the K+ equilibrium potential, E_K. This hyperpolarization would be expected to increase anion efflux from the CPe into the CSF. We found mildly (14%) increased CSF [Cl−] in Kcne2−/− mice compared with that in Kcne2+/+ mice (134±6 vs. 118±5 mM, respectively, measurements performed on 6 aliquots each from independent CSF samples of n=5 mice/genotype, P<0.05, 1-tailed Student’s t test). In contrast, there were no changes in serum [Cl−], CSF pH, or CSF [K+] apparent with Kcne2 deletion (Table 1).

**DISCUSSION**

KCNE2 and KCNQ1 were each originally described as subunits contributing to the two major human cardiac ventricular repolarization currents: KCNE2 in complex with human ether-a-go-go-related gene generating I_Kr and KCNQ1 in complexes with KCNE1 generating I_Ks (7). KCNQ1 and KCNE2 were later discovered to form a constitutively active channel together in vitro (20), and found to provide a parietal cell apical K+ efflux pathway essential for gastric acid
Figure 6. Kcne3 and Kcnq1 localization in Kcne2+/+ and Kcne2−/− mouse CPe. A) Immunofluorescence of embryonic Kcne2+/+ and Kcne2−/− mouse CPe, showing immunoreactivity with anti-Kcne2 antibody (E2, red) in Kcne2+/+ but not Kcne2−/− CPe; DAPI staining (D, blue). Representative of n = 3 tissue sections. B) Immunofluorescence of adult Kcne2+/+ and Kcne2−/− mouse CPe, showing immunoreactivity with anti-Kcnq1 antibody (Q1, red) in the apical membrane of Kcne2+/+ mouse CPe but in the basolateral and/or intracellular compartment of Kcne2−/− mouse CPe; DAPI staining (D, blue). Representative of n = 3 tissue sections. White arrows, apical membrane; yellow arrows, basal membrane. C) Immunofluorescence of adult Kcne2+/+ mouse CPe, showing apical localization of Kcne2 (E2, red) and Kcnq1 (Q1; green); white arrows indicate colocalization; DAPI staining (D, blue). Representative of n = 3 tissue sections. D) Immunofluorescence of adult Kcne2+/+ mouse CPe, showing signal for Kcnq1 (Q1, red) and NKCC1 (NK, green) only in the CPe, with no staining in neighboring neuronal tissue (bottom right, bright field image of the same region for comparison). White arrowhead, CPe. Representative of n = 2 tissue sections.

(continued on next page)
secretion (15, 23). The KCNQ1-KCNE2 channel is also expressed at the basolateral membrane of thyrocytes (19).

Unlike KCNQ1, KCNA3 retains its voltage dependence when coexpressed with KCNE2, but its currents are partially suppressed. As with some other KCNE2α subunit partnerships, there is cell type- and/or KCNE species-specific dependence to this interaction: in another recent study, human KCNE2 was found not to modulate rat Kcn3 function in HEK cells (21). Consistent with this finding, we previously found that rat KCNE2 functionally regulates KCNCl (Kv3.1) and KCNC2 (Kv3.2) in CHO cells, whereas human KCNE2 does not (24). The mechanistic basis for this difference is not yet known; one or more of the 24 nonconserved amino acid residues between rat and human KCNE2 (8) may underlie the disparity, or it could relate to cell-type specific regulatory processes we do not yet understand. The finding here that Kcne2 deletion shifts KCN3 and KCNQ1 trafficking to the CPe basolateral membrane recapitulates our recent finding of the same reversal in trafficking polarity of KCNQ1 in gastric parietal cells (25). The α subunit rerouting may be a determining factor in the CPe dysfunction in Kcne2−/− mice, although the simple increase in KCN3 and KCNQ1 outward currents could also explain the observed effects.

KCNQ1-KCNE2 and KCNA3-KCNE2 channels probably do not play a major part in regulating baseline CSF [K+]−. Kcne2 disruption here did not result in altered baseline CSF [K+]−, and the most important regulator of CSF [K+]− is probably active K+ removal from the CSF to the CPe by the Na+−/K+ ATPase, followed by transport from the CPe to the blood by KCC3 and perhaps KCCL1 and as yet unidentified basolateral K+ channels (3). However, although K+ must be removed from the CSF by apical CPe uptake followed by basolateral efflux, some K+ ions must also be returned to the CSF to prevent hypokalorrhachia (low CSF [K+]−), and the most likely conduits for this are the apical K+ channels (3). It is possible that KCNE2-containing channels are required for regulating CSF [K+]− when it has been acutely perturbed; furthermore, we only measured the [K+]− of cisternal CSF for accessibility reasons, but it is possible that the [K+]− of other, inaccessible CSF fractions is regulated by CPe KCNE2-containing channels.

These caveats notwithstanding, we hypothesize two functions for KCNQ1-KCNE2 and/or KCNA3-KCNE2 in the CPe. First, they may provide a K+ efflux pathway to counteract K+ influx through the apical Na+−/K+ ATPase, somewhat analogous to the role of KCNQ1-KCNE2 coupled to the gastric H+−/K+ ATPase in parietal cells. Second, KCNQ1-KCNE2 and KCNA3-KCNE2 may regulate Cl− secretion from the CPe to the CSF, as does the related KCNQ1-KCNE3 channel in the colon (26). In support of this hypothesis, we found that Kcne2 deletion increased outward K+ current, hyperpolarized the CPe, and increased CSF [Cl−]. The hyperpolarization would tend to drive out Cl− ions via apical channels such as the as-yet unidentified apical anion channel, tallying well with the increased CSF [Cl−] in Kcne2−/− mice (Table 1; Fig. 6J).

KCNQ1 is recognized primarily for its roles in the heart and polarized epithelia outside the CNS, although it was recently reportedly to be detected in forebrain neuronal networks and brainstem nuclei, where its precise function has not yet been ascertained (27). Mice with an LQTS-associated KCNQ1 variant knocked-in were found to have seizure-precipitated cardiac arrhythmic episodes, suggesting a

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TABLE 1. Effects of Kcne2 deletion on CPe and CSF parameters

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CPe Em (mV)</th>
<th>CSF pH</th>
<th>CSF [K+] (mM)</th>
<th>Serum [Cl−] (mM)</th>
<th>CSF [Cl−] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcne2+/+</td>
<td>−39.5 ± 1.5 (10)</td>
<td>7.5 ± 0.3 (10)</td>
<td>3.2 (9)</td>
<td>107 ± 1 (6)</td>
<td>118 ± 5 (5)</td>
</tr>
<tr>
<td>Kcne2−/−</td>
<td>−48.1 ± 1.9 (12)*</td>
<td>7.3 ± 0.4 (10)</td>
<td>3.1 (10)</td>
<td>111 ± 1 (6)</td>
<td>134 ± 6 (5)*</td>
</tr>
</tbody>
</table>

CPe and CSF parameters are indicated as means ± se, where available, with n values in parentheses; absence of se indicates that intragenotype samples were pooled before measurement. *P < 0.05 vs. Kcne2+/+.

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E) Merged, magnified view of adult Kcne2+/+ mouse CPe images from D, showing strong overlap of Kcnq1 (Q1, red) and Nkcc1 (NK, green), giving yellow signal. Representative of n = 2 tissue sections. F) Immunofluorescence of adult Kcne2+/+ mouse CPe, showing lack of colocalization of Kcnq1 (Q1, red) with the apically localized Nkcc1 (NK, green). Yellow arrowhead, punctate Kcnq1 staining; blue arrowhead, diffuse Kcnq1 staining. Representative of n = 3 tissue sections. G) Immunofluorescence of adult Kcne2−/− mouse CPe, showing colocalization of Kcnq1 (Q1, red) with Kcn3 (A3; green); Dapi staining (blue). Representative of n = 3 tissue sections. H) Immunofluorescence of adult Kcne2+/+ mouse CPe, showing colocalization of Nkcc1 (NK, green) with Kcn3 (A3; red) (white arrow). Representative of n = 2 tissue sections. I) Immunofluorescence of adult Kcne2−/− mouse CPe, showing lack of colocalization of Kcn3 (A3, red) with the apically localized Nkcc1 (NK, green); Dapi staining (D, blue). Representative of n = 3 tissue sections. J) Model suggesting the effect of Kcne2 deletion in the CPe. Kcne2 deletion increases currents though Kcnq1 and Kcn3 and switches them to the basolateral membrane. Increased K+ currents hyperpolarize the cell, possibly increasing Cl− efflux through apical anion channels (AC). KCC, K+−/Cl− cotransporter; NKA, Na+−/K+−ATPase; Nkcc, Nkcc1. Scale bars = 50 μm (A, B); 10 μm (C, F); 200 μm (D); 40 μm (E, I); 3 μm (G, H).
role for KCNQ1 in regulating neuronal excitability, and also a potential mechanistic basis for sudden unexplained death in epilepsy in humans (27). A role for KCNQ1 in regulating CSF composition, as we report here, raises questions as to whether this magnitude of altered CSF composition could also contribute to neuronal excitability disorders, for example, by shifting the resting membrane potential of the neurons or glial cells which it bathes. It also raises the issue of whether CPe KCNQ1-KCNE2 channels, despite the importance of KCNQ1 to human cardiac function, can ever constitute a therapeutic target for treating disorders of the nervous system involving deregulated CSF secretion and composition, including cerebral edema and intracranial hypertension. Although KCNQ1-KCNE1 channels are important for human ventricular myocyte repolarization and their loss of function linked to LQTS, KCNQ1-KCNE2 channels have a different pharmacological profile than KCNQ1-KCNE1 channels, including increased sensitivity to the Chromanol 293B-like molecule IKS124 (28). In addition, KCNQ1 agonists, which could potentially be used to hyperpolarize the CPe, would not necessarily be cardiotoxic as they would not be predicted to prolong the QT interval.

The recent discovery of a human KCNQ2 mutation in a case of neonatal seizures was intriguing and, if supported by further similar findings, could suggest a role in human inherited epilepsy (10), although how disruption of the function of KCNQ2 in the CPe membrane might potentially alter neuronal excitability remains unclear. Targeted deletion of the Slc4a10 gene, which encodes the Na+/HCO3⁻ exchanger Slc4a10, expressed on the CPe basolateral membrane, decreases seizure susceptibility and decreases basolateral Cl⁻ efflux; however, perturbations in CSF pH appeared more important in the decreased neuronal excitability in this case (29), whereas we did not observe changes in CSF pH with Kcne2 deletion (Table 1). It is important to note that although we have focused on the roles of KCNQ2 in the CPe because of its prominent expression there compared with that in the rest of the brain, the previous discoveries that KCNQ2 regulates KCNQ2-KCNQ3 and HCN (pacemaker) channels in vitro and that their neuronal expression potentially overlaps, for example, in the thalamus and hippocampus (13, 30, 31), suggest that KCNQ2 could also play significant roles directly in specific neurons.

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