Benign familial neonatal convulsions (BFNC), a class of idiopathic generalized epilepsy, is an autosomal dominantly inherited disorder of newborns. BFNC has been linked to mutations in two putative K⁺ channel genes, KCNQ2 and KCNQ3. Amino acid sequence comparison reveals that both genes share strong homology to Kv-LQT1, the potassium channel encoded by KCNQ1, which is responsible for over 50% of inherited long QT syndrome. Here we describe the cloning, functional expression, and characterization of K⁺ channel encoded by KCNQ2 and KCNQ3 cDNAs. Individually, expression of KCNQ2 or KCNQ3 in Xenopus oocytes elicits voltage-gated, rapidly activating K⁺-selective currents similar to KCNQ1. However, unlike KCNQ1, KCNQ2 and KCNQ3 currents are not augmented by coexpression with the KCNQ1 β subunit, KCNE1 (minK, IsK). Northern blot analyses reveal that KCNQ2 and KCNQ3 exhibit similar expression patterns in different regions within the brain. Interestingly, coexpression of KCNQ2 and KCNQ3 results in a substantial synergistic increase in current amplitude. Coexpression of KCNQ1 with the two channels strongly suppressed current amplitude and slowed kinetics of activation. The pharmacological and biophysical properties of the K⁺ currents observed in the coexpressed oocytes differ somewhat from those observed after injection of either KCNQ2 or KCNQ3 by itself. The functional interaction between KCNQ2 and KCNQ3 provides a framework for understanding how mutations in either channel can cause a form of idiopathic generalized epilepsy.

Potassium channels are the largest and most diverse group of ion channels. They are primary regulators of resting membrane potential and action potential configuration and, therefore, modulate excitability of neurons, cardiac myocytes, and other electrically active cells. Recent identification of KCNQ1 (KvLQT1), the gene responsible for more than 50% of inherited cardiac long QT syndrome (LQT3), established a new family of six-transmembrane domain K⁺ channels (1). KCNQ1, in combination with the KCNE1 subunit, encodes the slow component of the cardiac delayed rectifier K⁺ current (2–4), and mutations in KCNQ1, which occur in LQTS patients, partially or completely inhibit the channel in a dominant-negative fashion (5, 6). In an attempt to identify additional members of the KCNQ1 K⁺ channel gene family, the KCNQ1 sequence was used to search DNA and protein sequence data banks. Two additional KCNQ1-related genes, KCNQ2 and KCNQ3, were identified.

Recent publications indicate that mutations in KCNQ2 or KCNQ3 are associated with BFNC, an autosomal dominantly inherited epilepsy in newborns (7–9). Preliminary functional characterization of KCNQ2 confirmed that this gene encodes a voltage-activated K⁺ channel (9). Here we describe the cloning, tissue distribution, and functional expression of both KCNQ2 and KCNQ3. More importantly, we demonstrate that these two channels interact functionally with each other and with KCNE1.
Cloning and Tissue Distribution of KCNQ2 and KCNQ3—
KCNQ1-related expressed sequence tags (ESTs) were identified in a GCG BLAST search of the GenBank™ data base with KCNQ1 sequence. Primers, derived from the consensus sequences of EST clones, were used to amplify human brain-derived cDNA, and 877-base pair and 325-base pair fragments were isolated for KCNQ2 and KCNQ3, respectively. To obtain full-length cDNA sequences of both genes, we employed 5′ rapid amplification of cDNA ends polymerase chain reaction, screening of cDNA libraries, and Gene Trapper techniques. The composite full-length cDNAs of KCNQ2 and KCNQ3 contain an open reading frame (ORF) encoding an 871- and 854-amino acid polypeptide, respectively. DNA sequence analysis and conceptual translation of both cDNAs reveals that they encode proteins with the structural features of a voltage-gated potassium channel and are most closely related to KCNQ1 (3, 4). Both proteins have a longer C-terminal domain (>200 amino acids) than KCNQ1.

Unlike KCNQ1, which is expressed strongly in human heart and pancreas (1, 4), Northern blot analysis revealed that KCNQ2- and KCNQ3-specific transcripts are detectable only in human brain (Fig. 1). The sizes of the major transcripts for KCNQ2 and KCNQ3 are 8.5 kilobases and 10.5 kilobases, respectively. Expression of human KCNQ2 is high in the hippocampus, caudate nucleus, and amygdala; moderate in the thalamus, and weak in the subthalamic nucleus, substantia nigra, and corpus callosum (Fig. 1, top panel). A separate Northern blot demonstrates that expression of human KCNQ2 is high in the cerebral cortex, is moderate in the putamen, temporal lobe, frontal lobe, occipital pole, and cerebellum, and is barely detectable in the medulla and spinal cord (Fig. 1, top panel). A similar pattern of expression was observed previously for KCNQ2 (7). Interestingly, KCNQ3 exhibits a nearly identical expression pattern in the brain (Fig. 1, bottom panel).

Functional Expression and Characterization of KCNQ2 and KCNQ3—The full-length KCNQ2 and KCNQ3 cDNAs were subcloned into a Xenopus expression vector, and cRNA was generated by in vitro transcription. The properties of the channels encoded by KCNQ2 and KCNQ3 were investigated by expressing the transcribed cRNAs in Xenopus oocytes. Depolarizing voltage steps elicited outward currents in oocytes injected with KCNQ2 (Fig. 2A). The currents activated at potentials positive to −60 mV and showed slight inward rectification at the more positive potentials. Similar currents never were observed in water-injected control oocytes. KCNQ2 currents exhibited a rapidly activating delayed rectifier current phenotype similar to KCNQ1 current (2–4). Fig. 2B shows the current-voltage (I-V) relationship for KCNQ2 currents recorded at the end of the 1-s voltage steps. The K+ selectivity of the expressed current was examined by investigation of tail current reversal potentials in bath solutions containing 2, 10, 40, and 98 mM K+. Reversal potentials closely followed the Nernst potential for K+ revealing a predominantly K+-selective channel (Fig. 2C). The reversal potential for KCNQ2 current shifted by 51 mV per 10-fold change in external K+.

Inhibitors of K+ channels were used to investigate the pharmacology of KCNQ2. The effects of 4-AP, E-4031, clofilium, charybdotoxin (CTX), and TEA on KCNQ2 currents recorded from a single oocyte are shown in Fig. 2D. Each of these compounds also was tested alone in individual oocytes, and the effects of each agent were consistent with the data shown in Fig. 2. CTX (100 nM), a protein from scorpion venom that inhibits a variety of Ca2+-activated and voltage-dependent K+ channels (10, 11), did not inhibit KCNQ2 current. CTX also had no effect on KCNQ1 current (not shown). E-4031 (10 μM), a
Functional Expression of KCNQ2 and KCNQ3

A family of currents elicited by depolarizing voltage steps in an oocyte injected with KCNQ3 cRNA are shown in Fig. 2E. The currents activate at potentials positive to −70 mV and rectify inwardly at potentials greater than 0 mV, as is obvious from the I−V relationship (Fig. 2F). The KCNQ3 reversal potential shifted 49 mV per 10-fold change in external K+ concentration. The dashed line has a slope of 58 mV and is drawn according to the Nernst equation for a perfectly selective K+ channel. Each value is the mean ± S.E. from 6 oocytes.

B. Current-voltage (I−V) relationship for oocytes expressing KCNQ2 (n = 6). Currents were recorded using the protocol in A. C. Dependence of tail current reversal potential (Erev) on the external K+ concentration. The dashed line has a slope of 58 mV and is drawn according to the Nernst equation for a perfectly selective K+ channel. Each value is the mean ± S.E. from 6 oocytes.

D. Effects of E-4031, 4-AP, TEA, charybdotoxin, and clofilium on KCNQ2 current. Superimposed currents were recorded during 1-s steps to −80 mV, from a holding potential of −110 to +110 mV, to test potentials ranging from −70 to +50 mV in 10-mV increments. B, current-voltage (I−V) relationship for oocytes expressing KCNQ2 (n = 6). Currents were recorded using the protocol in A. C, dependence of tail current reversal potential (Erew) on the external K+ concentration. The dashed line has a slope of 58 mV and is drawn according to the Nernst equation for a perfectly selective K+ channel. Each value is the mean ± S.E. from 6 oocytes. D, effects of E-4031, 4-AP, TEA, charybdotoxin, and clofilium on KCNQ2 current. Superimposed currents were recorded during 1-s steps to +20 mV, from −80 mV, during the same experiment. Compounds were applied via bath perfusion in order from top to bottom. The bath was perfused with control solution for 5 min or until effects reversed completely, between compounds. Similar results were obtained in three additional oocytes. E, families of currents from KCNQ3 cRNA-injected oocytes elicited using the protocol in A. F, I−V relationship for oocytes expressing KCNQ3 (n = 6). G, dependence of tail current Erew on the external K+ calculated using the protocol in C (n = 6). H, effects of E-4031, 4-AP, TEA, and clofilium on KCNQ3 current. Similar results were obtained in three additional oocytes.

selective inhibitor of the HERG K+ channel (12), and 4-AP (2 mM), an inhibitor of Shaker-type K+ channels (13), also had no significant effects on KCNQ2 current. Similarly, neither agent inhibits KCNQ1 channels (4). Clofilium (10 µM), a compound that inhibits KCNQ1 (4) with an IC50 <10 µM, had little effect on KCNQ2 current. TEA (1 mM), a nonselective K+ channel inhibitor and weak KCNQ1 antagonist (4), reduced KCNQ2 current by 90%.

A family of currents elicited by depolarizing voltage steps in an oocyte injected with KCNQ3 cRNA are shown in Fig. 2E. The currents activate at potentials positive to −70 mV and rectify inwardly at potentials greater than 0 mV, as is obvious from the I−V relationship (Fig. 2F). The KCNQ3 reversal potential shifted 49 mV per 10-fold change in external K+ (Fig. 2G). Thus, although still predominantly selective for K+, KCNQ3 is slightly less K+-selective than KCNQ2. The pharmacology of KCNQ3 was significantly different from that of KCNQ2 (Fig. 2H). Clofilium (10 µM) reduced KCNQ3 current by 30% from control but had little effect on KCNQ2. TEA, which strongly inhibited KCNQ2 at 1 mM, produced little inhibition of KCNQ3 at 5 mM. CTX (100 nM; not shown), 4-AP (2 mM), and E-4031 (10 µM) also had no effect on KCNQ3 current. Thus, although both KCNQ2 and KCNQ3 encode related voltage-activated K+ channels, significant differences include: (a) degree of rectification at positive voltages, (b) minimum activation voltage, (c) selectivity for K+, and (d) pharmacology.

KCNQ2 and KCNQ3 Functionally Interact—The overlapping expression pattern of KCNQ2 and KCNQ3 in different brain regions (Fig. 1), together with the fact that mutations in either KCNQ2 or KCNQ3 cause the same inherited epilepsy (BFNC; 7–9), prompted us to test for functional interaction between the two channels. Families of currents elicited by depolarizing voltage steps in oocytes injected with KCNQ2 and KCNQ3 alone and together are shown in Fig. 3A. Current amplitudes recorded from oocytes coexpressing the two channels were 15-fold greater than in oocytes injected with each of the channels individually. Peak current amplitudes at +30 mV for KCNQ2, KCNQ3, and KCNQ2+KCNQ3 were 0.98 ± 0.09 (n = 6), 0.98 ± 0.06 (n = 5), and 14.2 ± 0.62 µA (n = 6), respectively. Quantitatively similar results were obtained in three separate batches of oocytes. The I−V relationship shows that KCNQ2+KCNQ3 currents activated at potentials positive to −60 mV and did not rectify, unlike KCNQ2 and particularly KCNQ3, at positive voltages (Fig. 3B). The reversal potential of tail currents shifted by 57 mV per 10-fold change in external K+ indicating that KCNQ2+KCNQ3 is nearly perfectly selective for K+ (Fig. 3C). KCNQ2+KCNQ3 current is weakly sensitive to inhibition by 5 mM TEA and 10 µM clofilium but not to 100 mM CTX or 2 mM 4-AP (Fig. 3D). E-4031 (10 µM) also did not inhibit KCNQ2+KCNQ3 current (not shown). These results suggest strongly that KCNQ2+KCNQ3 interact to form a channel with properties distinct from either KCNQ2 or KCNQ3 channels.

mink Interacts with KCNQ2+KCNQ3 Channels—The β subunit KCN1 dramatically alters the amplitude and gating kinetics of the KCN1 channel (2–4, 14). Because KCNQ2 and KCNQ3 are members of the same K+ channel subfamily, we
tested for an interaction between KCNE1 and KCNQ2+KCNQ3 channels. Fig. 4 shows currents elicited by 1-s depolarizing voltage steps from a holding potential of −80 mV, to test potentials ranging from −70 to +50 mV (10-mV increments). Effects of 4-AP, TEA, charybdotoxin, and clofilium on KCNQ2+KCNQ3 current. Superimposed currents were recorded during 1-s steps to +20 mV, from −80 mV, during the same experiment. Compounds were applied via bath perfusion in order from top to bottom. Similar results were obtained in 4 additional oocytes.
brain which coexpress KCNQ2 and KCNQ3 also express KCNE1 remains to be determined. The effect of KCNE1 on gating kinetics is similar for KCNQ1 and KCNQ2+KCNQ3 channels. In contrast, KCNE1 augments KCNQ1 current but inhibits KCNQ2+KCNQ3. Mutations in KCNE1 cause LQT5 and produce dominant-negative suppression of KCNQ1 current (15). Although KCNE1 has opposite effects on KCNQ1 and KCNQ2+KCNQ3 channels, it will be interesting to determine whether mutations in KCNE1 account for altered neuronal excitability.

The results explain why mutations in either of two unlinked K^+-channel encoding genes yield the same phenotype. BFNC-associated mutations in either KCNQ2 or KCNQ3 could cause a profound reduction in KCNQ2+KCNQ3 current amplitude. Interestingly, a BFNC-causing mutation resulting in a non-functional, truncated KCNQ2 protein, failed to produce a dominant-negative inhibition of wild-type KCNQ2 channels expressed in oocytes (7). Our results, demonstrating a synergistic interaction between KCNQ2 and KCNQ3, may provide a likely explanation for this finding. That is, mutations in KCNQ2 may only produce dominant-negative effects when coexpressed with wild-type KCNQ3 channels and vice versa. This supports the suggestion from the previous study (7) that dominant-negative effects of KCNQ2 mutants may require a β-subunit or second protein. This information will prove important for the evaluation of functional effects of channel mutations that cause BFNC and perhaps other disorders of neuronal excitability.

Acknowledgments—We thank B. Kienzle for DNA sequencing, T. Jenkins-West for oocyte preparation and expert technical assistance, and W. Koster and D. Hathaway for discussion, comments, and support.

REFERENCES
1. Wang, Q., Curran, M. E., Splawski, I., Burns, T. C., Millholland, J. M., VanRaay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., Jager, T. D., Schwartz, P. J., Towbin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Conners, T. D., and Keating, M. T. (1996) Nat. Genet. 12, 17–23
2. Barhanin, J., Lesage, F., Guillermare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) Nature 384, 78–80
3. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80–83
4. Yang, W.-P., Levesque, P. C., Little, W. A., Conder, M. L., Shalaby, F. Y., and Blianar, M. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4017–4021
5. Chouabe, C., Neyroud, N., Guicheney, P., Lazdunski, M., Romey, G., and Barhanin, J. (1997) EMBO J. 16, 5472–5479
6. Shalaby, F. Y., Levesque, P. C., Yang, W.-P., Little, W. A., Conder, M. L., Jenkins-West, T., and Blianar, M. A. (1997) Circulation 96, 1733–1736
7. Biervert, C., Schroeder, B. C., Kubisch, C., Berkovic, S. F., Propping, P., Jentsch, T. J., and Steinlein, O. K. (1998) Science 279, 403–406
8. Charlier, C., Singh, N. A., Ryan, S. G., Lewis, T. B., Reus, B. E., Leach, R. J., and Leppert, M. (1998) Nat. Genet. 18, 53–55
9. Singh, N. A., Charlier, C., Stauffer, D., DuPont, B. R., Leacj, R. J., Melis, R., Ronen, G. M., Bjerre, I., Quattlebaum, T., Murphy, J. Y., McHarg, M. L., Gagnon, D., Rosales, T. O., Pfeffer, A., Anderson, V. E., and Leppert, M. (1998) Nat. Genet. 18, 25–29
10. Sugg, E. E., Garcia, M. L., Reuben, J. P., Patchett, A. A., and Kaczorowski, G. J. (1999) J. Biol. Chem. 265, 18745–18748
11. Miller, C., Moczydlowski, E., Lotorre, R., and Phillips, M. (1985) Nature 313, 316–318
12. Snyders, D. J., and Chaudhary, A. (1996) Mol. Pharmacol. 49, 949–955
13. Deal, K. K., England, S. K., and Tamkun, M. M. (1996) Physiol. Rev. 76, 49–67
14. Romey, G., Attali, B., Chouabe, C., Abitbol, I., Guillermare, E., Barhanin, J., and Lazdunski, M. (1997) J. Biol. Chem. 272, 16713–16716
15. Splawski, I., Tristani-Firouzi, M., Lehmann, M. H., Sanguinetti, M. C., and Keating, M. T. (1997) Nat. Genet. 17, 338–340