Benign Familial Neonatal Convulsions Caused by Altered Gating of KCNQ2/KCNQ3 Potassium Channels

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The muscarinic-regulated potassium current (M-current), formed by the heteromeric assembly of subunits encoded by the *KCNQ2* and *KCNQ3* genes, is a primary regulator of neuronal excitability; this regulation is accomplished by impeding repetitive firing and causing spike-frequency adaptation. Mutations in *KCNQ2* or *KCNQ3* cause benign familial neonatal convulsions (BFNC), a rare autosomal-dominant generalized epilepsy of newborns, by reducing the maximal current carried by the M-channels without affecting ion selectivity or gating properties. Here we show that KCNQ2/KCNQ3 channels carrying a novel BFNC-causing mutation leading to an arginine to tryptophan substitution in the voltage-sensing S_4 domain of KCNQ2 subunits (R214W) displayed slower opening and faster closing kinetics and a decreased voltage sensitivity with no concomitant changes in maximal current or plasma membrane expression. These results suggest that mutation-induced gating alterations of the M-current may cause epilepsy in neonates.

Key words: benign familial neonatal convulsions; BFNC; muscarinic regulated potassium current; M-current; potassium channel gating; S_4 voltage sensor; KCNQ2; epilepsy

Voltage-dependent potassium (K^+) channels represent the most heterogeneous class of ion channels with respect to kinetic properties, regulation, pharmacology, and structure (Shieh et al., 2000). In the nervous system, voltage-dependent K^+ channels play a crucial role in regulating neuronal excitability by controlling action potential duration, subthreshold electrical properties, and responsiveness to synaptic inputs.

The muscarinic-regulated K^+ current (M-current), first described in peripheral neurons (Brown and Adams, 1980) and subsequently in the CNS (Halliwell and Adams, 1982), is a widespread regulator of neuronal excitability. In fact, during long-lasting depolarizing inputs, this slowly activating and noninactivating current tends to repolarize the neuronal membrane back toward resting membrane potential, thus limiting repetitive firing and causing spike-frequency adaptation (Rogawski, 2000). Receptor-dependent suppression or stimulation of the M-current is therefore a primary mechanism by which neurotransmitters and neuromodulators may enhance or blunt, respectively, neuronal excitability (Marrion, 1997). The molecular identity of the M-channels has remained elusive until the recent demonstration that it is primarily formed by the heteromeric assembly of K^+ channel subunits encoded by the *KCNQ*2 and *KCNQ3* genes (Wang et al., 1998; Cooper et al., 2000), although other members of the *KCNQ* subfamily, namely *KCNQ4* (Kubisch et al., 2000) and *KCNQ5* (Lerche et al., 2000; Schroeder et al., 2000), may contribute to M-current heterogeneity.

The fundamental role played by the M-current in the control of neuronal excitability in humans has received strong genetic support from the discovery that mutations in either *KCNQ2* (Biervert et al., 1998; Singh et al., 1998) or *KCNQ3* (Charlier et al., 1998) are responsible for benign familiar neonatal convulsions (BFNC), a rare autosomal-dominant idiopathic epilepsy of the newborn. This disease is characterized by the occurrence of multifocal or generalized tonic–clonic convulsions starting at approximately day 3 of postnatal life and spontaneously disappearing after a few weeks or months (Steinlein, 1998). Although neurocognitive development is normal in most BFNC-affected individuals, 10–15% of them will experience convulsive manifestations later in life (Ronen et al., 1993).

The use of heterologous expression systems to examine the functional effects of the BFNC-causing mutations is crucial for understanding the pathogenesis of the disease and for interpret-

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ing its dominant mode of inheritance. The results obtained up to today (Biervert et al., 1998; Schroeder et al., 1998; Lerche et al., 1999; Schwake et al., 2000) suggest that most of these mutations cause a mild reduction in the maximal current carried by the KCNQ2/KCNQ3 M-channels, with no significant dominant-negative effects or changes in channel gating or ion selectivity.

In this report, we describe the study of the functional consequences of a novel BFNC-causing mutation in *KCNQ2* (c686t) found in a large, four-generation Italian family leading to an arginine to tryptophan substitution in the S_4 voltage-sensing domain of KCNQ2 subunits (R214W) (Miraglia del Giudice et al., 2000). The present electrophysiological and biochemical results, showing that the R214W mutation in KCNQ2 altered the gating properties of the M-channels without affecting their maximal current or plasma membrane expression, suggest that mutation-induced gating alterations of the M-current may cause epilepsy in neonates.

MATERIALS AND METHODS

Isolation of Xenopus *oocytes.* The dissociation, maintenance, and microinjection of *Xenopus* oocytes followed standard procedures (Taglialatela et al., 1997). Briefly, ovarian lobes were surgically removed from adult female *Xenopus* frogs and individual oocytes were dissociated by enzymatic treatment with collagenase (type IA; 2 mg/ml) for 45–80 min in a Ca^{2+}-free solution. In the experiments described in Figure 3*C*, the follicular layer was removed manually (Schwake et al., 2000). Once dissociated, Ca^{2+} was reintroduced in the oocyte-bathing solution and the oocytes were stored in a 19°C incubator for use on the following day.

Mutagenesis and oocyte injection. KCNQ2 and KCNQ3 cDNAs were cloned in pTLN vectors as described previously (Schroeder et al., 1998). Mutations in KCNQ2 were engineered by sequence-overlap extension PCR with the *Pfu* DNA polymerase, using a *Not*I–*Pml*I cassette. DNA sequences were verified by manual sequencing. After linearization, plasmids were transcribed *in vitro* with a commercially available kit (mCAP; Stratagene, La Jolla, CA) using the SP6 RNA polymerase. RNAs were quantified using the RiboGreen RNA quantification kit (Molecular Probes, Leiden, The Netherlands) and stored at 150–250 ng/μl at −20°C in 0.1 M KCl. *Xenopus* oocytes were microinjected with 50 nl of cRNA diluted to achieve the desired concentration.

Electrophysiology. At 2–7 d after cRNA microinjection, expressed K^+ currents were measured at room temperature with the two-microelectrode voltage-clamp technique. The extracellular recording solution contained (in mM) 96 NaCl, 2 KCl, 2.6 $MgCl_2$, 0.18 $CaCl_2$, and 5 HEPES, pH 7.5. pClamp software (version 6.0.2; Axon Instruments, Foster City, CA) was used for data acquisition and analysis.

Surface expression of wild-type and mutant KCNQ2 subunits. Surface expression was measured by inserting a hemagglutinin (HA) epitope into the extracellular S_1–S_2 linker of Q2 and Q2R214W subunits (Schwake et al., 2000). With expression of the HA-tagged constructs, plasma membrane surface expression was measured by incubating intact oocytes with a rat anti-HA antibody followed by a horseradish peroxidase-coupled goat anti-rat secondary antibody. After 1 min of incubation in power signal ELISA solution, the chemiluminescence of individual oocytes was quantified with a Lumat LB 9507 luminometer (EG&G Berthold, Bad Wildbad, Germany) as described previously (Zerangue et al., 1999).

Data analysis and statistics. Activation and deactivation kinetics were calculated by fitting the current records to a sum of two exponential functions, as already reported for KCNQ2/KCNQ3 channels and native M-current (Wang et al., 1998), where τ_f and τ_s are the time constants of the fast and slow exponentials, respectively. Conductance–voltage curves were generated by normalizing to the maximal value the tail currents recorded during repolarization to −70 mV and expressing the normalized values as a function of the preceding voltages. As described for *Shaker* K^+ channels (Stefani et al., 1994), the data were fit to a sum of two independent Boltzmann distributions (B_1 and B_2) of the following form: $y = max_1/[1 + exp(V_1 - V)/k_1] + max_2/[1 + exp(V_2 - V)/k_2]$, where V is the test potential, V_1 and V_2 are the half-activation potentials, k_1 and k_2 are the slopes, max_1 and max_2 are the maximal amplitudes for each Boltzmann distribution, and *exp* is the exponential function (power of *e*, the base of natural logarithms). IC_{50} values for extracellular TEA (TEA_e) blockade were calculated by fitting the percentage of inhibition of the currents elicited by 1.75 sec depolarizations to +20 mV by the different TEA concentrations (from 0.01 to 30 mM) to the following binding isotherm: $y = max/(1 + X/IC_{50})$, where X is the TEA_e (Taglialatela et al., 1991). Statistically significant differences between the data were evaluated with the Student's t test. Data are expressed as the mean ± SEM.

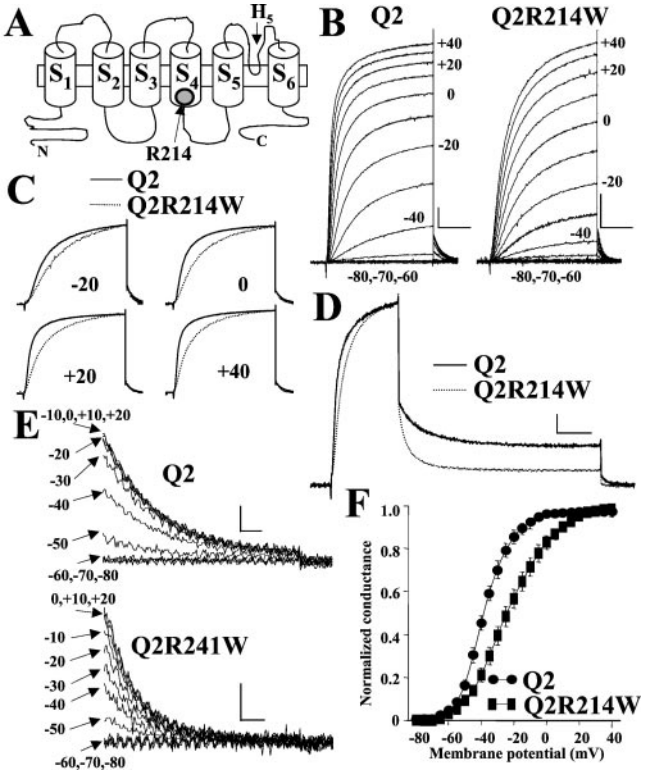

Figure 1. Effect of the R214W mutation on homomeric KCNQ2 channel function. *A*, Putative transmembrane topology of a KCNQ2 subunit. The arginine at position 214 in S_4 is indicated by a *gray circle*. H_5 denotes the pore region. *B*, Current traces from *Xenopus* oocytes injected with 5 ng/oocyte of Q2 or Q2R214W cRNAs in response to membrane depolarizations of 10 mV increments from −80 to +40 mV. Holding potential, −90 mV. Calibration: 200 nA, 500 msec. *C*, Comparison between the activation kinetics of Q2 and Q2R214W homomeric channels after appropriate scaling. *D*, Deactivation kinetics of Q2 and Q2R214W homomeric channels. Holding potential, −90 mV; 1 sec depolarizing pulse to +20 mV, followed by a 3 sec repolarization to −50 mV. Calibration: 10% of the peak current at +20 mV (scaling as in *C*), 500 msec. *E*, Tail currents from Q2 or Q2R214W channels recorded during repolarization to −70 mV after 1.75 sec depolarizations to the potentials indicated by the *arrows*. *F*, Voltage dependence of the normalized conductance of Q2 and Q2R214W channels. The *solid lines* represent the fits of the experimental data to the sum of two independent Boltzmann distributions (see Materials and Methods).

RESULTS

Expression of homomeric KCNQ2 R214W channels

Functional voltage-dependent K^+ channels assemble as tetramers of identical (homomers) or compatible (heteromers) subunits, each displaying six putative transmembrane segments and a pore-forming H_5 domain (Shieh et al., 2000). The fourth transmembrane segment (S_4) contains between four and eight basic residues spaced by hydrophobic amino acids and is thought to form a major part of the voltage sensor (Bezanilla, 2000). Figure 1*A* shows the putative topological arrangement of a single KCNQ2 (Q2) subunit. R214 is the innermost of the six arginine residues in S_4 and is highly conserved in the KCNQ K^+ channel subfamily;

Table 1. Gating and pharmacological properties of channels carrying KCNQ2 mutations found in BFNC patients

RNA injected			Activation						Deactivation		TEA_e block
Type	Amount/oocyte (ng)	n	τ_f (msec)	τ_s (msec)	V_1 (mV)	V_2 (mV)	k_1 (mV/*e*-fold)	k_2 (mV/*e*-fold)	τ_f (msec)	τ_s (msec)	IC_{50} (mM)
Q2	5	9	61 ± 4	412 ± 20	−42.3 ± 0.5	−24.9 ± 2.2	6.1 ± 0.2	8.3 ± 0.5	121 ± 14	513 ± 51	0.13 ± 0.01
Q2R214W	5	9	154 ± 26*	639 ± 43*	−34.9 ± 0.7*	−9.5 ± 2.2*	8.3 ± 0.4*	12.3 ± 0.6*	84 ± 7*	354 ± 38*	0.12 ± 0.01
Q2Y284C	5	5	67 ± 6	444 ± 44	ND	ND	ND	ND	ND	ND	109 ± 9*
Q2/Q3	0.25/0.25	10	74 ± 7	441 ± 20	−43.6 ± 0.6	−29.8 ± 0.9	5.6 ± 0.1	6.3 ± 0.2	137 ± 4	721 ± 44	1.4 ± 0.1
Q2R214W/Q3	0.25/0.25	9	159 ± 13#	1175 ± 258#	−37.3 ± 0.5#	−17.8 ± 2.3#	6.6 ± 0.3#	10.3 ± 0.5#	88 ± 7#	442 ± 31#	1.1 ± 0.1
Q2/Q2R214W/Q3	0.125/0.125/0.25	7	114 ± 18#	726 ± 153#	−40.6 ± 0.6#	−20.1 ± 1.1#	6.6 ± 0.4#	10.4 ± 0.6#	103 ± 2#	518 ± 17#	1.3 ± 0.1
Q2/Q2Y284C/Q3	0.25/0.25/0.5	7	83 ± 5	432 ± 35	ND	ND	ND	ND	ND	ND	39 ± 0.3#

n, Number of cells recorded; ND, not detected; *$p < 0.05$ versus Q2; #$p < 0.05$ versus Q2/Q3.

the only exception is KCNQ1, where a glutamine residue is present at the corresponding position (Q244).

To assess the functional consequences of the KCNQ2 R214W (Q2R214W) mutation found in the BFNC-affected family, we heterologously expressed the mutant channel subunits in *Xenopus* oocytes. Homomeric channels composed of mutant Q2R214W subunits displayed activation kinetics much slower than those of homomeric Q2 channels (Fig. 1*B,C*); at +20 mV, both the fast and slow time constants of activation (τ_f and τ_s, respectively) (Wang et al., 1998) were faster in homomeric Q2 channels with respect to homomeric Q2R214W channels (Table 1). In addition, the ratio of the relative amplitudes of the fast and slow activation components (A_f and A_s, respectively), expressed as $A_f/A_f + A_s$, was greater in Q2 than in Q2R214W homomeric channels (64.5 ± 3% versus 49.6 ± 4.2%, respectively; $p < 0.05$). Interestingly, the activation kinetics of homomeric channels carrying another mutation found in BFNC-affected families, Y284C, located in the H_5 region of KCNQ2 (Q2Y284C), were identical to those of homomeric Q2 channels (Table 1).

Introduction of the R214W mutation in KCNQ2 channels not only slowed the activation kinetics but also increased the rate of channel closing (Fig. 1*D,E*); in fact, at −50 mV, both the fast and slow deactivation time constants of homomeric Q2R214W were faster than those of Q2 channels (Table 1). In addition, the ratio of the relative amplitudes of the two deactivation components, expressed as $A_f/A_f + A_s$, was 31.4 ± 2.5% and 59.9 ± 4.1% in Q2 and Q2R214W homomeric channels ($p < 0.05$). As a result, the percentage of current at the end of the 3 sec −50 mV pulse relative to the peak current at +20 mV was decreased from 27.6 ± 3.4% in Q2 homomeric channels to 8.6 ± 1.6% in Q2R214W homomeric channels ($p < 0.05$). Furthermore, the voltage dependence of homomeric Q2R214W channels was more shallow and shifted toward more depolarized voltages when compared with homomeric Q2 channels (Fig. 1*E,F*; see below).

Expression of KCNQ2 R214W subunits in heteromeric channels with KCNQ3 or KCNQ2/KCNQ3 subunits

To mimic the genetic condition of BFNC-affected patients, who carry a single mutated *KCNQ2* allele, and considering that KCNQ2 subunits are thought to assemble with KCNQ3 (Q3) subunits to form the M-current (Wang et al., 1998; Cooper et al., 2000), coexpression experiments of Q2R214W subunits with Q3 (1:1 ratio) and with Q2 plus Q3 subunits (0.5:0.5:1 ratio) were also performed.

Similarly to homomeric Q2R214W channels, heteromeric channels composed of Q2R214W/Q3 or Q2/Q2R214W/Q3 subunits displayed slower activation when compared with Q2/Q3 channels (Fig. 2*A,B*); however, expression of Q2Y284C mutant subunits together with Q2/Q3 (0.5:0.5:1 ratio) failed to affect the Q2/Q3 channel activation kinetics (Table 1). Furthermore, at −50 mV, deactivation τ_f and τ_s were slower in Q2/Q3 channels when compared with either Q2R214W/Q3 or Q2/Q2R214W/Q3 channels (Table 1). In addition, the relative weight of the fast deactivation component, expressed as $A_f/A_f + A_s$, was 33.7 ± 4.4% for Q2/Q3 channels, 50.6 ± 1.1% for Q2R214W/Q3 channels ($p < 0.05$ vs Q2/Q3), and 49.8 ± 1.9% for Q2/Q2R214W/Q3 channels ($p < 0.05$ vs Q2/Q3). As a result, the percentage of current at the end of the −50 mV repolarizing pulse relative to the peak current at +20 mV was 24.6 ± 1.2% in Q2/Q3 channels, 14.3 ± 2.5% in Q2R214W/Q3 channels ($p < 0.05$ vs Q2/Q3), and 18.7 ± 0.4% in Q2/Q2R214W/Q3 channels ($p < 0.05$ vs Q2/Q3). Also, in close analogy to the effects observed in the homomeric configuration, heteromeric assembly of Q2R214W subunits with Q3 or with

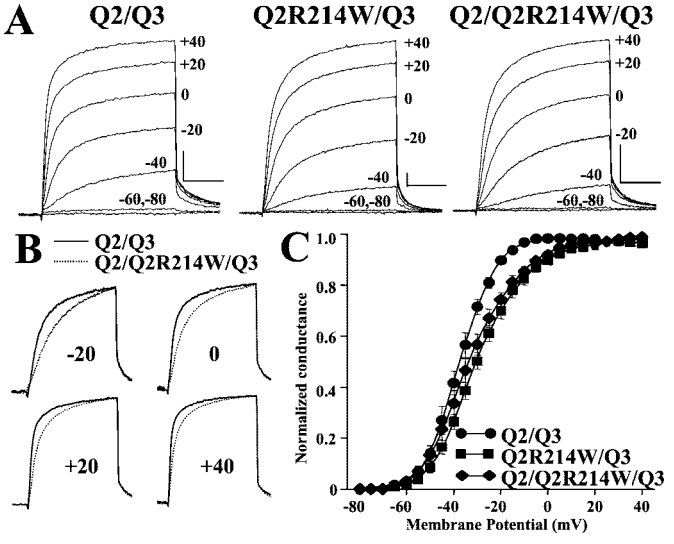

Figure 2. KCNQ2 R214W subunit expression in heteromeric channels with KCNQ3 or KCNQ2/KCNQ3. *A*, Current traces recorded during depolarization to the indicated voltages of *Xenopus* oocytes injected with Q2 and Q3 cRNAs (0.25 + 0.25 ng/oocyte, respectively); Q2R214W and Q3 cRNAs (0.25 + 0.25 ng/oocyte, respectively); or Q2, Q2R214W, and Q3 cRNAs (0.125 + 0.125 + 0.25 ng/oocyte, respectively). Calibration: 100 nA, 500 msec. *B*, Comparison of the activation kinetics of heteromeric Q2/Q3 and Q2/Q2R214W/Q3 channels after appropriate scaling. *C*, Voltage dependence of the conductance of heteromeric Q2/Q3, Q2R214W/Q3, and Q2/Q2R214W/Q3 channels. The *solid lines* represent the fits of the experimental data to the sum of two independent Boltzmann distributions (see Materials and Methods).

Q2/Q3 subunits decreased the steepness and caused a rightward shift in the voltage dependence of channel activation (Fig. 2*C*; see below).

Interestingly, the gating changes introduced during incorporation of the R214W mutation in KCNQ2 observed in both homomeric and heteromeric configurations were not accompanied by changes in the sensitivity to blockade by TEA_e (Table 1); in contrast, the Q2Y284C mutation, both in homomeric and heteromeric configurations with Q3 or Q2/Q3 subunits, disrupted the high-affinity binding site for TEA (Taglialatela et al., 1991; Heginbotham and MacKinnon, 1992). Also, the high channel selectivity for K^+ over Na^+ was retained with the expression of Q2R214W subunits; in fact, with 2 mM extracellular K^+, the reversal potentials of the expressed currents (in mV) were -92.3 ± 2.8 ($n = 6$), -93.8 ± 1.3 ($n = 6$), -96.6 ± 2.8 ($n = 5$), and -95.6 ± 2.2 ($n = 5$) for homomeric Q2, homomeric Q2R214W, heteromeric Q2/Q3, and heteromeric Q2/Q2R214W/Q3 channels, respectively.

The R214W mutation decreases the voltage dependence of channel activation

Closer inspection into the voltage dependence of activation of Q2 homomeric channels (Fig. 1*F*) and Q2/Q3 heteromeric channels (Fig. 2*C*) revealed that the sum of two Boltzmann distributions (Stefani et al., 1994), one with steeper voltage dependence activating at more negative voltages (B_1), and one more shallow activating at more depolarized potentials (B_2), was required to adequately fit the experimental data. The R214W substitution in KCNQ2, expressed in homomeric or heteromeric configurations with Q3 or Q2/Q3 subunits, significantly affected the midpoint potentials (V_1, V_2) and the slopes (k_1, k_2) of both Boltzmann distributions (Table 1). Quantitatively larger effects of the R214W substitution were observed on the transitions occurring at more depolarized membrane voltages. Comparing the data obtained during triple coexpression of Q2/Q2R214W/Q3 subunits with those of Q2/Q3, we found that the B_2 component was shifted by 10 mV toward more depolarized potentials and had a 4 mV/*e*-fold decrease in slope (*e* is the base of natural logarithms, and is $\simeq 2.72$.) In contrast, B_1 was only shifted by 3 mV and had a 1 mV/*e*-fold decrease in slope. In addition, the relative contributions of the two Boltzmann components (max_1 and max_2 for B_1 and B_2, respectively) were 67.7 ± 5.3% and 29.4 ± 5.4% for homomeric Q2 channels, 48.9 ± 5.7% and 50.4 ± 5.6% for homomeric Q2R214W channels ($p < 0.05$ vs Q2), 46.6 ± 5.7% and 51.9 ± 5.3% for heteromeric Q2/Q3 channels, 54.5 ± 7.2% and 42.5 ± 7.4% for heteromeric Q2R214W/Q3 channels ($p > 0.05$ vs Q2/Q3), and 54.6 ± 7.8% and 43.7 ± 7.2% for heteromeric Q2/Q2R214W/Q3 channels ($p > 0.05$ vs Q2/Q3).

The KCNQ2 R214W mutation does not reduce the maximal current carried by the M-channels

Given the significant changes in channel voltage-dependent gating promoted by the KCNQ2 R214W substitution, we studied the effects of this mutation on the maximal current and on the plasma membrane expression of the mutant channel subunits to investigate whether the gating changes observed were the only functional defects induced by the mutation.

The maximal current carried by the channels incorporating Q2R214W subunits in homomeric (Fig. 3*A*) or heteromeric configuration with Q3 or Q2/Q3 subunits (Fig. 3*B*) was identical to that carried by homomeric Q2 or heteromeric Q2/Q3 channels, respectively. In contrast, and in agreement with previous studies

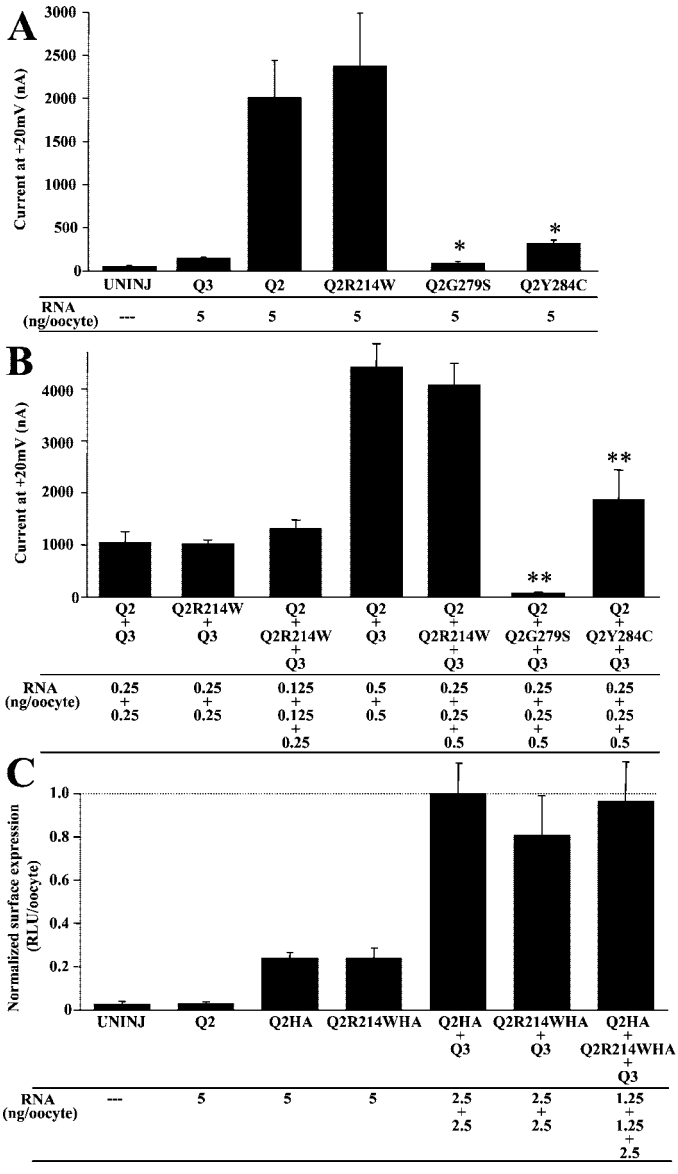

Figure 3. Maximal current and plasma membrane expression of homomeric or heteromeric channels containing KCNQ2 R214W mutant subunits. The maximal current recorded in *Xenopus* oocytes expressing wild-type Q3, Q2, Q2R214W, Q2G279S, or Q2Y284C subunits in homomeric (*A*) or heteromeric (*B*) configurations is shown. Each *bar* is the mean ± SEM of the peak current recorded in 10–25 oocytes (three to five batches from different donor frogs) at the end of a 1.75 sec pulse to +20 mV. $^*p < 0.05$ versus Q2 (5 ng/oocyte); $^{**}p < 0.05$ versus Q2/Q3 (0.5 + 0.5 ng/oocyte, respectively). *C*, Plasma membrane expression of Q2 and Q2R214W subunits. Data are expressed as relative luminescence units (*RLU*) per oocyte (10 sec reading), after normalization to those of the Q2HA/Q3 group (15,760,210 ± 2,750,169 RLU/oocyte). Each bar is the mean ± SEM of 12–28 oocytes (three batches from different donor frogs).

(Schroeder et al., 1998), the Y284C BFNC-causing mutation in KCNQ2 reduced the maximal current in both homomeric or heteromeric configurations with Q2/Q3, although to a lesser degree than the strong dominant-negative G279S pore mutation in KCNQ2 (Schroeder et al., 1998; Schwake et al., 2000) (Fig. 3*A,B*).

Biochemical measurements of surface expression of the mutated subunits (Zerangue et al., 1999; Schwake et al., 2000) showed that the surface levels of KCNQ2 subunits carrying the R214W substitution were identical to those of wild-type KCNQ2

subunits in both homomeric and heteromeric configurations (Fig. 3*C*), a result that is consistent with the lack of effect of the R214W substitution on the maximal amount of current carried by channels incorporating the mutated subunits.

DISCUSSION

Epilepsy is a disorder of recurrent episodes of aberrant hyperexcitability in neuronal networks that affects ~0.5% of the population (Noebels, 1996). Although convulsive diseases caused by single-gene disorders only account for a minority of idiopathic epilepsies in humans, the identification and the study of the functional consequences of the genetic alterations underlying familiar epilepsies is crucial for clarifying the pathophysiology of the disease, for unraveling the role played by the altered genes, and for designing novel therapeutic approaches (Steinlein, 1998). Among monogenic epilepsies, BFNC represents so far one of the best recognized disease models of generalized idiopathic epilepsies (Hirsch et al., 1999); therefore, investigation of the molecular mechanisms by which the genetic alterations found in affected patients cause BFNC is of fundamental relevance also for the treatment of generalized idiopathic epilepsies in the adult population.

The present study has been performed to elucidate the functional consequences of a novel BFNC-causing missense mutation leading to the replacement of the innermost basic residue with a neutral residue in the S_4 voltage-sensing domain of KCNQ2 subunits (R214W) (Miraglia del Giudice et al., 2000). KCNQ2 subunits are crucial (Wang et al., 1998; Cooper et al., 2000) but not exclusive (Kubisch et al., 2000; Lerche et al., 2000; Schroeder et al., 2000) determinants of the M-current, a widely distributed K^+-selective current exerting inhibitory control on neuronal excitability.

The results obtained suggest that the primary mechanism for the altered neuronal excitability in BFNC-affected patients carrying the KCNQ2 R214W mutation is a change in the gating properties of the M-channels. In fact, heterologous expression of KCNQ2 R214W subunits, both in homomeric or heteromeric configurations with KCNQ3 or KCNQ2 plus KCNQ3 subunits, led to the appearance of macroscopic currents having slower activation, faster deactivation, and decreased voltage sensitivity. These changes in gating were not accompanied by significant changes in the permeation and blocking properties, because the K^+/Na^+ selectivity ratio and the sensitivity to TEA_e block remained unaffected during homomeric or heteromeric expression of KCNQ2 R214W subunits. In contrast, and in agreement with previous studies (Schroeder et al., 1998; Schwake et al., 2000), another mutation found in BFNC-affected families, Y284C, localized in the pore-forming H_5 region of KCNQ2, failed to affect M-current gating properties, but it abolished channel sensitivity to TEA_e block. The opposite effects exerted on gating and pore properties by the two mutations found in BFNC patients provide strong support for a modular structure of K^+ channels where the gate and the pore, although functionally coupled, are structurally distinct.

Coexpression experiments also allowed investigation of the molecular basis for the dominant mode of BFNC inheritance. In fact, assuming equal translation capacity of the injected cRNAs (see below), triple coinjection at the 0.5:0.5:1 ratio of KCNQ2 R214W, KCNQ2, and KCNQ3 cRNAs, which is an experimental strategy designed to mimic the heterozygotic condition of BFNC-affected patients, should result in a majority of tetrameric channels carrying a single mutated subunit. The persistence of significant gating alterations in KCNQ2/KCNQ2 R214W/KCNQ3 triple heteromeric channels, when compared with KCNQ2 R214W/KCNQ3 channels (carrying two mutated subunits) or homomeric KCNQ2 R214W channels (having four mutated subunits), indicates that the substitution of the innermost arginine in the S_4 voltage sensor of a single KCNQ2 subunit was sufficient to introduce slower rate-limiting steps in the chain of events leading to channel opening (Bezanilla, 2000). This might explain the dominant mechanism of inheritance of BFNC in patients carrying the KCNQ2 R214W mutation.

Previous studies on the functional consequences of the BFNC-associated mutations in KCNQ2 or KCNQ3 have suggested that the K^+ channel subunits carrying mutations causing extensive deletions in the C terminus did not form functional channels and did not reach the plasma membrane (Biervert et al., 1998; Schwake et al., 2000); however, subunits carrying missense mutations in the core domain (from S_1 through S_6) can assemble normally but function less efficiently (Schroeder et al., 1998; Lerche et al., 1999; Schwake et al., 2000), as revealed by a 20–40% maximal current reduction. These results, confirmed by the present experiments with the KCNQ2 Y284C mutation, have led to the conclusion that a mild decrease in M-channel maximal current is a primary pathophysiological mechanism for BFNC caused by missense mutations.

Interestingly, in these studies, the gating properties of the channels carrying the mutant subunits were unaffected. In contrast, the changes in gating described here for the channels incorporating the R214W mutation in KCNQ2 occurred in the absence of modifications in either maximal current or plasma membrane expression. This suggests that changes in M-channel gating may lead to BFNC. In fact, the slower activation and faster deactivation kinetics, coupled with a decreased voltage sensitivity caused by the R214W mutation in the KCNQ2/KCNQ3 heteromeric M-channels, could effectively decrease the ability of this widespread regulator of neuronal excitability to dampen epileptiform discharges in several regions of juvenile brains. The present results are therefore consistent with the idea that the gating alterations prompted by the KCNQ2 R214W mutation cause BFNC because of a decreased neuronal repolarization reserve mediated by the M-current (Rogawski, 2000).

Finally, the altered gating properties induced by the R214W mutation in KCNQ2 might also have considerable implications for genotype-specific therapy. In fact, the novel anticonvulsant molecule retigabine (Main et al., 2000; Wideckenden et al., 2000) shifts the voltage dependence of the M-channels toward more hyperpolarized voltages, a phenomenon opposite to that caused by the R214W mutation investigated here. Thus, it seems likely that the functional consequences of the mutation might influence the antiepileptic efficacy of the drug.

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