Three New Familial Hemiplegic Migraine Mutants Affect P/Q-type Ca\textsuperscript{2+} Channel Kinetics*

Richard L. Kraus, Martina J. Sinnegger†, Alexandre Koschak, Hartmut Glossmann, Stefania Stenirri§, Paola Carrera§, and Jörg Striessnig†

From the Institut für Biochemische Pharmakologie, Peter-Mayr-Strasse 1, Innsbruck A-6020, Austria, and the Clinical Molecular Biology Laboratory, Hospital San Raffaele, Milan 20132, Italy

Missense mutations in the pore-forming human α\textsubscript{1A} subunit of neuronal P/Q-type Ca\textsuperscript{2+} channels are associated with familial hemiplegic migraine. We studied the functional consequences on P/Q-type Ca\textsuperscript{2+} channel function of three recently identified mutations, R583Q, D715E, and V1457L after introduction into rabbit α\textsubscript{1A} and expression in Xenopus laevis oocytes. The potential for half-maximal channel activation of Ba\textsuperscript{2+} inward currents was shifted by > 9 mV to more negative potentials in all three mutants. The potential for half-maximal channel inactivation was shifted by > 7 mV in the same direction in R583Q and D715E. Biexponential current inactivation during 3-s test pulses was significantly faster in D715E and slower in V1457L than in wild type. Mutations R583Q and V1457L delayed the time course of recovery from channel inactivation. The decrease of peak current through R583Q (30.2%) and D715E (30.1%) but not V1457L (18.7%) was more pronounced during 1-Hz trains of 15 100-ms pulses than in wild type (18.2%). Our data demonstrate that the mutations R583Q, D715E, and V1457L, like the previously reported mutations T666M, V714A, and I1811L affect P/Q-type Ca\textsuperscript{2+} channel gating. We therefore propose that altered channel gating represents a common pathophysiological mechanism in familial hemiplegic migraine.

Voltage-gated P/Q-type Ca\textsuperscript{2+} channels are expressed on cell bodies and dendrites of cerebellar Purkinje cells and other neurons (1–3) where they are thought to control neuronal excitability, gene expression, neuronal plasticity, and differentiation. These channels are also expressed on presynaptic terminals (3) mediating depolarization-induced Ca\textsuperscript{2+} influx tightly coupled to neurotransmitter release (4). The Ca\textsuperscript{2+}-selective pore of P/Q-type Ca\textsuperscript{2+} channels is formed by α\textsubscript{1A} subunits, which also contain the voltage sensors. α\textsubscript{1A} is encoded by the human gene CACNA1A on chromosome 19p13 (5).

P/Q-type Ca\textsuperscript{2+} channels have received much attention recently because CACNA1A mutations have been described which are responsible for at least three different neurological human diseases: episodic ataxia type 2 (EA-2), spinocerebellar ataxia type 6, and familial hemiplegic migraine (FHM) with and without cerebellar ataxia. These mutations may provide important insight into how altered Ca\textsuperscript{2+} signaling and neuronal excitability can lead to neurodegeneration and episodic neurological diseases such as migraine.

Four nonsense mutations (6–8), three splice site mutations, and four deletions in CACNA1A (5, 7) have been found to segregate in patients with EA-2. Small CAG expansions were observed in a large series of patients with spinocerebellar ataxia type 6 (9), and a further CACNA1A missense mutation was identified in a patient with severe progressive ataxia (10). At least seven missense mutations have been identified in families with FHM (5, 11–13). Defects in the α\textsubscript{1A} gene are also responsible for the phenotypes (absence epilepsy and ataxia) of tootering (tg) and leaner (tg\textsuperscript{e}) mouse mutants and may also occur in more common forms of migraine (15).

The mechanisms by which these mutations cause these abnormal phenotypes is unclear. Mutations causing EA-2, an autosomal dominant disease, are predicted to give rise to truncated, presumably nonfunctional α\textsubscript{1A} proteins. This must result in a partial loss of P/Q-type Ca\textsuperscript{2+} channel function.

In contrast, we (16) and others (17) have shown recently that α\textsubscript{1A} missense mutations causing FHM do not prevent channel activity. FHM is a rare autosomal dominant form of migraine with aura, associated with icctal hemiparesis and, in some families, with cerebellar ataxia and atrophy (18). Functional expression of rabbit α\textsubscript{1A} subunits containing the FHM mutations T666M, V714A, and I1811L revealed mutation-induced changes in gating kinetics altering the extent to which P/Q-type channels accumulate in inactivation during trains of depolarizing pulses. We therefore proposed that this could alter Ca\textsuperscript{2+} influx and signaling during episodes of high neuronal activity. This in turn might result in a long term activation of neurons within the proposed “migraine generator” in the brainstem discovered by brain imaging in migraine patients (19).

Essentially the same changes in gating kinetics were reported by Hans et al. (17) after introduction of the same FHM mutations in human α\textsubscript{1A} followed by heterologous expression in human embryonic kidney 293 cells and patch clamp analysis. In addition, they reported mutation-induced changes in single channel kinetics and expression density.

In the current study we examined the functional effects of three recently published FHM mutations, R583Q, D715E, and V1457L (11–13) to address further the important questions of whether all FHM mutations yield functional Ca\textsuperscript{2+} channels

* This work was supported in part by Fonds zur Förderung der Wissenschaftlichen Forschung Grants P-12641 (to J. S.) and P-12689 (to H. G.) and by the Österreichische Nationalbank (to J. S.), the Dr. Legerlotz Foundation, and the University of Innsbruck. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of a Hertha-Firnberg fellowship.

‡ To whom correspondence should be addressed. Tel.: 43-512-507-3164; Fax: 43-512-588-627; E-mail: joerg.striessnig@uibk.ac.at.

§ The abbreviations used are: EA-2, episodic ataxia type 2; FHM, familial hemiplegic migraine; BAPTA, 1,2-bis(2-aminophenoxo)ethane-N,N,N’,N’-tetraacetic acid; I\textsubscript{Ba}, inward Ba\textsuperscript{2+} currents; V\textsubscript{0.5,act}, half-maximal voltage for activation; k\textsubscript{act}, slope factor of the curve at V\textsubscript{0.5,act}; V\textsubscript{0.5,inact}, half-maximal voltage for steady-state inactivation; k\textsubscript{inact}, slope factor of the curve at V\textsubscript{0.5,inact}.

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
and if altered channel gating represents a key pathophysiologi-
ical principle in FHM as proposed from initial studies.

EXPERIMENTAL PROCEDURES

Mutant α1A cDNAs—Mutations were introduced into rabbit class A Ca\(^{2+}\) channel α1A cDNA (BI-II, 1) by applying the “gene-SOEing” tech-
ique (20) as described previously (21). Nucleotide positions of endonu-
clease restriction sites are given in parentheses. α1A mutants were cloned into the polyadenylating transcription plasmids pcDNA3.1 (1) or pNF9240 (a gift of O. Pongs). Single mutants R583Q and D715E were constructed according to the previously described procedure for generation of single mutants T6466M and V714A (16).

Mutation V1457L (corresponding to V1465L in rabbit α1A; see legend to Fig. 1) as well as a silent mutation (codon for rabbit Asp-1545 GAC to GAT) were introduced simultaneously into rabbit class A cDNA by polymerase chain reaction to yield a ClaI restriction sequence. The mutated polymerase chain reaction fragment was cut Sfil (4290)-ClaI (4295) and coligated with a Nbhi (3543)-Sfil (4290) fragment of BI-II into AL20 Nbhi (3543)-ClaI (homologous position to ClaI(+)) (22) to yield complete rabbit α1A cDNA sequence. All polymerase chain reaction-generated fragments were sequenced completely to confirm sequence integrity.

Expression of α1A Mutants in Xenopus laevis Oocytes—Preparation of stage V–VI oocytes from X. laevis and injection of cRNA are described in detail elsewhere (21). Capped run-off poly(A)\(^{+}\) cRNA transcripts from XbaI-linearized cDNA templates were synthesized according to the procedures of Krieg and Melton (23). α1A cRNAs were coinjected with β1a (24) and α2δ (25) subunit cRNAs. To exclude effects of endogenous Ca\(^{2+}\)-activated Cl\(^{-}\) currents on current kinetics, experiments were carried out in oocytes previously injected with 50–100 nl of a 0.1 mM BAPTA solution.

Electrophysiological Recordings—Inward Ba\(^{2+}\) currents (I\(_{\text{Ba}}\)) through expressed channel complexes were measured using the two-microelectrode voltage clamp technique as described previously (21). Similar current amplitudes were obtained with mutant and wild-type α1A subunits. Oocytes expressing peak I\(_{\text{Ba}}\) smaller than 400 nA or larger than 1.5 \(\mu\)A were excluded from analysis. Data analysis and acquisition were performed by using the PClamp software package (version 6.0, Axon Instruments).

Recordings were carried out at room temperature in a bath solution containing 40 mM Ba(OH)\(_2\), 50 mM NaOH, 2 mM CsOH, 5 mM HEPES, adjusted to a pH of 7.4 with methanesulfonic acid. Voltage recording and current injecting microelectrodes were filled with 2.8 M CsCl, 0.2 M CsOH, 10 mM EGTA, 10 mM HEPES (adjusted to pH 7.4 with HCl) and had resistances of 0.3–2 megohm.

Recovery of I\(_{\text{Ba}}\) from inactivation was studied using a double-pulse protocol. After a 3-s depolarization to +10 mV (holding potential) to –80 mV, the time course of I\(_{\text{Ba}}\) recovery was determined at –60 mV by applying 300-ms test pulses to +10 mV at various time intervals after the prepulse. Peak I\(_{\text{Ba}}\) was normalized to the peak current amplitude measured during the prepulse. I\(_{\text{Ba}}\) was then allowed to recover for 1 min at –100 mV. This double-pulse protocol was repeated individually for each recovery time interval in the same oocyte.

The voltage dependence of inactivation (steady-state inactivation) was determined from normalized inward currents elicited during steps to +10 mV after 10-s steps to various holding potentials. The voltage dependence of activation was determined from I-V curves obtained by step depolarizations from a holding potential of –80 mV to various test potentials. The half-maximal voltage for activation (V\(_{0.5,\text{act}}\)), the slope factor of the curve (k\(_{\text{act}}\)), and the slope factor of the curve (k\(_{\text{inh}}\)) were obtained by fitting the data to the Boltzmann equation. Apparent reversal potentials were calculated by extrapolation from I-V relationships.

Data Analysis—Nonlinear least square fitting and statistical calcu-
lations were performed using Origin\(^{\text{\textregistered}}\) 5.0 (Microcal). Data are given as means ± S.E. for the indicated number of experiments.

RESULTS

Mutations R583Q, D715E, and V1457L, illustrated in Fig. 1A, are located in highly conserved and functionally important regions of the human α1A subunit of neuronal P/Q-type Ca\(^{2+}\) channels. Mutation R583Q neutralizes a positive charge in transmembrane segment S4 of the channel in domain II (IIS4). S4 segments form part of the voltage sensor of voltage-gated Ca\(^{2+}\) channels (26). D715E is located in IIS6 adjacent to mutation V1414A analyzed in our previous study and V1457L in the S5–S6 linker of domain III. Segments S5 and S6 and their connecting linkers are assumed to form the pore of the channel (26). We introduced the single mutations into the corresponding positions of the highly homologous rabbit α1A subunit (wild type, Ref. 1) and analyzed mutant channels for changes in their biophysical properties after functional expression in X. laevis oocytes (together with accessory β1 and α2δ subunits) using the two-microelectrode voltage clamp technique.

The potential for half-maximal activation (V\(_{0.5,\text{act}}\)) was sig-
ificantly (p < 0.01) shifted to hyperpolarized potentials for all three mutants without changing the steepness of the steady-
state activation curve (Table I). This effect was most pro-
nounced in D715E. The midpoint voltage for steady-state inac-
vitation was not altered in mutant V1457L, but a significant shift to more negative potentials occurred in R583Q and D715E (Table I). Apparent reversal potentials were similar for all constructs (53–59 mV) ruling out major changes in Ba\(^{2+}\) permeability.

To investigate whether the FHM mutations affect the time
course of channel inactivation we analyzed the current decay during 3-s test pulses elicited from a holding potential of −80 mV to +10 mV (Fig. 1B). For wild-type and mutant channels the time course of inactivation could be well described by a double-exponential function. D715E significantly (p < 0.01) accelerated both the time constant for the initial fast component (τfast = 0.142 ± 0.004 s, n = 15) and the slow component (τslow = 0.577 ± 0.008 s, n = 15) of current decay compared with wild type (τfast = 0.227 ± 0.013 s; τslow = 0.806 ± 0.076 s, n = 5) (Fig. 1C). Mutation V1457L increased the time constant for the slow component of the current decay (τslow = 1.2 ± 0.033 s, n = 11). In R583Q τfast was also slightly accelerated, but this did not reach the level of statistical significance. The contribution of the fast component (wild type: 43.8 ± 6.6%; n = 5) was increased significantly in D715E (57.8 ± 6.5%; n = 14; p < 0.01) and decreased in V1457L (27.4 ± 6.5%; n = 10, p < 0.01).

Next we tested whether the mutations also change the extent of peak IBa decrease during pulse trains which reflects accumulation of channels in inactivation. Application of 15 100-ms pulses from a holding potential of −60 mV to a test potential of +10 mV at a frequency of 1 Hz caused a significant (p < 0.01) increase of accumulation in inactivation for mutants R583Q and D715E but not V1457L. Current decay after 15 pulses was 1.6-fold larger in R583Q (30.2 ± 0.8%; n = 35) and D715E (30.1 ± 1.5%; n = 18) than in wild type (19.1 ± 1%; n = 19). The fraction of channels inactivating during frequent depolarizations not only depends on the inactivation rate during the pulses but also on the rate of recovery from inactivation between pulses. Therefore recovery from inactivation was measured employing a double-pulse protocol (Fig. 3A). Channels were inactivated by a 3-s conditioning prepulse from −80 to +10 mV. The time course of recovery was then determined at −60 mV by applying 300-ms test pulses to +10 mV after various time intervals after the prepulse (Fig. 3A). Between single double-pulse experiments the oocytes were held at −100 mV for 60 s to allow full recovery of IBa. Recovery was determined at −60 mV to maximize the difference between wild-type and mutant channels. In wild-type and mutant channels about 90% of IBa recovered after 20 s. In all constructs recovery of IBa followed a biexponential time course (Fig. 3B). In both R583Q and V1457L the fraction of recovered current at all time intervals measured was significantly smaller (p < 0.01) than in wild type. No change was observed for D715E (Fig. 3B).

In summary, our experiments convincingly show that all newly discovered α1A mutations in patients with FHM cause abnormal gating behavior of P/Q-type Ca2+ channels. Gating changes therefore seem to represent an elementary mechanism underlying P/Q-type Ca2+ channel dysfunction in FHM.

**DISCUSSION**

We have studied the functional consequences of three recently identified FHM missense mutations, R583Q, D715E, and V1457L within the α1A subunit of neuronal P/Q-type Ca2+ channels. None of the mutations resulted in a nonfunctional channel as proposed for EA-2 mutations in the α1A subunit gene. EA-2 mutations (5, 7) are believed to be incompatible with the expression of a functional protein. In the presence of an unaffected gene, it is therefore likely that the observed
neurological phenotype in EA-2 results from a reduced activity of P/Q-type Ca$^{2+}$ channels in the central nervous system. Instead, two independent mechanisms can affect P/Q-type currents in FHM patients: altered expression density and changes in channel gating. Hans et al. (17) have recently found that FHM mutations decrease or increase the density of functional P/Q-type currents after heterologous expression in Xenopus oocytes or mammalian cells. It is difficult to predict if these changes of expression density also occur in vivo where, in addition to the accessory $\alpha_2\delta$ and $\beta$ subunits, $\alpha_1A$ interacts with a number of other modulatory proteins such as G-proteins (27), calmodulin (28), and synaptic vesicle proteins (29). Clearly, this important question can only be addressed in animal models containing the respective mutations.

A second mechanism by which FHM mutations can affect P/Q-type Ca$^{2+}$ currents is by changing channel gating. Our electrophysiological analysis provides convincing evidence that such changes also occur in three recently identified FHM mutations. Together with our previous results (16) this allows us to conclude that, irrespective of changes in expression density, this represents an elementary functional alteration underlying P/Q-type Ca$^{2+}$ channel dysfunction in FHM.

As for T666M, V714A, and I1811L, all three new mutations significantly shifted the voltage dependence of activation to more negative potentials. In the absence of changes in the slope of the activation curve this must result in a more negative threshold of Ca$^{2+}$ channel activation. This could lead to altered Ca$^{2+}$ signaling by increasing P/Q-type Ca$^{2+}$ channel activity at weak depolarizations. Two of the mutations also caused a more pronounced decrease of $I_{Na}$ during pulse trains, reflecting altered accumulation of channels in inactivation. This can result from either increased inactivation during the pulse or delayed recovery from inactivation between pulses. Our experiments demonstrate that it is due to slower recovery from inactivation in R583Q and faster inactivation in D715E. In V1457L decrease of $I_{Na}$ during the train was not different from wild type. This can be explained by the slower inactivation kinetics, which are counteracted by the slowed recovery from inactivation. Altered accumulation of channels in inactivation during rapid depolarizations could cause changes in Ca$^{2+}$ influx especially during high but not during low neuronal activity. This may underlie the episodic character of FHM with attacks triggered by sensory or emotional stimuli.

In addition to the potential insight into the pathophysiology of migraine, mutations R583Q and D715E also provide us with interesting molecular information about channel function. As in the previously analyzed mutant R192Q (16), R583Q eliminates a conserved positive charge at the extracellular side of transmembrane S4-helix, which forms part of the voltage sensor of the channel. The charge neutralization at position 583 in IIS4 (R583Q) shifted the voltage dependence of activation (and inactivation) to more negative potentials and slowed recovery from inactivation. These findings indicate that not only mutations in the putative pore region (T666M, V714A, I1811L) but also in the S4 segments can alter $\alpha_1A$ recovery from inactivation. This illustrates that conformational changes of voltage-sensing portions of $\alpha_1A$ are involved in this process.

Mutation R583Q caused a negative shift of $V_{0.5, act}$ without a change in the apparent gating charge, $z_p$ (Table I). Based on a simplified model describing the gating of a channel with only two states (open and closed) (Equation 2-22 in Ref. 30; 31) a negative shift of $V_{0.5, act}$ suggests that this mutation decreases the conformational energy difference between the closed and open states.

By assuming a model in which the voltage sensors in all four repeats move independently it can be predicted that Arg-583 (in IIS4) forms part of a voltage sensor which moves over potentials close to those causing channel opening. This is in contrast to data reported earlier for an $\alpha_1S$ (skeletal muscle)/$\alpha_1C$ (cardiac muscle) chimera where this was observed for sensors in repeats I and III but not in repeat II. Therefore this naturally occurring mutation clearly demonstrates that voltage sensor movements vary not only between different voltage-dependent cation channels (31) but even between different Ca$^{2+}$ channel $\alpha_1$ subunits.

Mutation D715E is located adjacent to mutation V714A. Together with I1811L in IVS6 these are believed to be located close to the cytoplasmic mouth of the pore. Unlike V714A and I1811L, V714E did not affect recovery from inactivation and prominently accelerated current inactivation upon depolarization. These data indicate that the cytoplasmic end of S6 helices comprise a functionally relevant region within $\alpha_1A$ which tightly controls the channel’s inactivation properties. Although our data do not allow us to propose a defined molecular mechanism for this process they clearly show that even minor structural changes such as the introduction of a single side chain methyl group in mutant D715E are sufficient to disturb this functional domain.

The present work clearly shows that mutations in the human CACNA1A gene alter the gating properties of neuronal P/Q-type Ca$^{2+}$ channels in all seven FHM mutants analyzed so far.
This provides a rational basis for the generation of mutant mice containing selected mutations. Introduction of FHM mutations differing with respect to their biophysical properties should enable electrophysiological analysis of the consequences of altered channel gating for neuronal Ca\(^{2+}\) signaling in FHM and more common forms of migraine (15).

Acknowledgments—We thank E. Wappi and E. Emberger for help in construction of mutants and P. Dietl for critical comments on the manuscript.

REFERENCES

1. Mori, Y., Friedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuschi, T., Mikoshiba, K., Imoto, K., Tanabe, T., and Numa, S. (1991) *Nature* **350**, 388–402

2. Stea, A., Tomlinson, W. J., Soong, T. W., Bourinet, E., Dubel, S. J., Vincent, S. R., and Snutch, T. P. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10576–10580

3. Westenbroek, R. E., Sakurai, T., Elliott, E. M., Hell, J. W., Starr, T. V., Snutch, T. P., and Catterall, W. A. (1995) *J. Neurosci.* **15**, 6403–6418

4. Wheeler, D. B., Tsien, R. W., and Randall, A. (1994) *Science* **266**, 828–831

5. Ophoff, R. A., Terwindt, G. M., van Eijk, R., Bulman, D. E., Hoffmann, S. M. G., Deonna, T., Gerard, P., Devoize, J. L., Gayou, A., Perrouty, B., Soisson, T., Autret, A., Warter, J. M., Vighetto, A., Van Bogaert, P., Alamovitch, S., Tournier-Lasserve, E., and Tournier-Lasserve, E. (1999) *Neurology* **53**, 1078–1087

6. Yue, Q., Jen, J. C., Thwe, M. M., Nelson, S. F., and Baloh, R. W. (1998) *Am. J. Hum. Genet.* **62**, 68–69

7. Denier, C., Ducros, A., Vahedi, K., Joutel, A., Thierry, P., Ritz, A., Castelnovo, M., Guerouaou, D., Tison, F., Julien, J., Hirsch, E., Chedru, F., Bisgard, C., Lucotte, G., Despres, P., Billard, C., Barthex, M. A., Ponsot, G., Bousser, M. G., and Tournier-Lasserre, E. (1999) *Am. J. Hum. Genet.* **64**, 89–98

8. Battistini, S., Stenirri, S., Piatti, M., Gelfi, C., Rigetti, P. G., Rocchi, R., Giannini, F., Battistini, N., Gazzuoli, G. C., Ferrari, M., and Carrera, P. (1999) *Neurology* **53**, 38–43

9. Carrera, P., Piatti, M., Stenirri, S., Grimaldi, L. M. E., Marchioni, E., Cureis, M., Rigetti, P. G., Ferrari, M., and Gelfi, C. (1999) *Neurology* **53**, 26–32

10. Fletcher, C. F., Lutz, C. M., O’Sullivan, T. N., Shaughnnessy, J. D., Hawkes, R., Frankel, W. N., Copeland, N. G., and Jenkins, N. A. (1996) *Cell* **87**, 607–617

11. Ducros, A., Denier, C., Joutel, A., Vahedi, K., Michel, A., Darcel, F., Madigand, M., Guerouaou, D., Tison, F., Julien, J., Hirsch, E., Chedru, F., Bisgard, C., Lucotte, G., Despres, P., Billard, C., Barthex, M. A., Ponsot, G., Bousser, M. G., and Tournier-Lasserre, E. (1999) *Am. J. Hum. Genet.* **64**, 89–98

12. Battistini, S., Stenirri, S., Piatti, M., Gelfi, C., Rigetti, P. G., Rocchi, R., Giannini, F., Battistini, N., Gazzuoli, G. C., Ferrari, M., and Carrera, P. (1999) *Neurology* **53**, 38–43

13. Carrera, P., Piatti, M., Stenirri, S., Grimaldi, L. M. E., Marchioni, E., Cureis, M., Rigetti, P. G., Ferrari, M., and Gelfi, C. (1999) *Neurology* **53**, 26–32

14. Fletcher, C. F., Lutz, C. M., O’Sullivan, T. N., Shaughnnessy, J. D., Hawkes, R., Frankel, W. N., Copeland, N. G., and Jenkins, N. A. (1996) *Cell* **87**, 607–617

15. May, A., Ophoff, R. A., Terwindt, G. M., Urban, C., van Eijk, R., Haan, J., Diener, H. C., Lindhout, D., Frants, R. R., Sandkuijl, L. A., and Ferrari, M. D. (1995) *Hum. Genet.* **96**, 604–608

16. Kraus, R. L., Sinnegger, M. J., Glossmann, H., Hering, S., and Stiessnig, J. (1998) *J. Biol. Chem.* **273**, 5386–5390

17. Hans, M., Lusisietto, S., Williams, M. E., Spagnolo, M., Urrutia, A., Tottene, A., Brust, P. L., Johnson, E. C., Harpold, M. M., Stauferman, K. A., and Pietrobon, D. (1997) *J. Neurosci.* **19**, 1610–1619

18. Terwindt, G. M., Ophoff, R. A., Haan, J., Vergouwe, M. N., van Eijk, R., Frants, R. R., and Ferrari, M. D. (1998) *Neurology* **50**, 1105–1110

19. Diener, H. C., and May, A. (1998) *Curr. Opin. Neurol.* **9**, 199–201

20. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) *Gene (Amst.)* **77**, 61–68

21. Grabner, M., Wang, Z., Hering, S., Stiessnig, J., and Glossmann, H. (1996) *Neurology* **52**, 207–218

22. van Eijk, R., Bulman, D. E., Hoffmann, S. M. G., Deonna, T., Campbell, K. P., McKenna, E., Koch, W. J., Hui, A., Schwartz, A., and Harpold, M. (1988) *Science* **241**, 1115–1118

23. Ellis, S. B., Williams, M. E., Ways, N. R., Brenner, R., Sharp, A. H., Leung, A. T., Campbell, K. P., McKenna, E., Koch, W. J., Hui, A., Schwartz, A., and Harpold, M. M. (1988) *Science* **241**, 1661–1664

24. Armstrong, C., and Hille, B. (1998) *Neuron* **20**, 371–380

25. Flockerzi, V., and Hofmann, F. (1989) *Science* **245**, 24471–24475

26. Krieg, P. A., and Melton, D. A. (1984) *Nucleic Acids Res.* **12**, 7057–7070

27. Ruth, P., Roehrkasten, A., Biel, M., Bosse, E., Regulla, S., Meyer, H. E., Flockerzi, V., and Hofmann, F. (1989) *Science* **245**, 24471–24475

28. Lee., A., Wong, S. T., Gallagher, D., Li, B., Storm, D. R., Scheuer, T., and Flockerzi, V. (1998) *Am. J. Hum. Genet.* **63**, 398–402

29. Rettig, J., Sheng, Z.-H., Kim, D. K., Hodson, C. D., Snutch, T. P., and Catterall, W. A. (1996) *J. Biol. Chem.* **271**, 24471–24475

30. Herlitze, S., Garcia, D. E., Mackie, K., Hille, B., Scheuer, T., and Catterall, W. A. (1996) *Nature* **380**, 258–262

31. Lee., A., Wong, S. T., Gallagher, D., Li, B., Storm, D. R., Scheuer, T., and Catterall, W. A. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7363–7368

32. Hille, B. (1992) *Neurology* **42**, 187–195

33. Garcia, J., Nakai, J., Imoto, K., and Beam, K. G. (1997) *Biophys. J.* **72**, 2315–2323
Three New Familial Hemiplegic Migraine Mutants Affect P/Q-type Ca$^{2+}$ Channel Kinetics
Richard L. Kraus, Martina J. Sinnegger, Alexandra Koschak, Hartmut Glossmann, Stefania Stenirri, Paola Carrera and Jörg Striessnig

*J. Biol. Chem. 2000, 275:9239-9243.*

doi: 10.1074/jbc.275.13.9239

Access the most updated version of this article at [http://www.jbc.org/content/275/13/9239](http://www.jbc.org/content/275/13/9239)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 31 references, 14 of which can be accessed free at [http://www.jbc.org/content/275/13/9239.full.html#ref-list-1](http://www.jbc.org/content/275/13/9239.full.html#ref-list-1)