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Communication

A Novel K^+ Channel β-Subunit (hKvβ1.3) Is Produced via Alternative mRNA Splicing*

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Voltage-gated K^+ channels can form multimeric complexes with accessory β-subunits. We report here a novel K^+ channel β-subunit cloned from human heart, hKvβ1.3, that has 74–83% overall identity with previously cloned β-subunits. Comparison of hKvβ1.3 with the previously cloned hKvβ3 and rKvβ1 proteins indicates that the carboxyl-terminal 328 amino acids are identical, while unique variable length amino termini exist. Analysis of human β-subunit cDNA and genomic nucleotide sequences confirm that these three β-subunits are alternatively spliced from a common β-subunit gene. Co-expression of hKvβ1.3 in *Xenopus* oocytes with the delayed rectifier hKv1.5 indicated that hKvβ1.3 has unique functional effects. This novel β-subunit induced a time-dependent inactivation during membrane voltage steps to positive potentials, induced a 13-mV hyperpolarizing shift in the activation curve, and slowed deactivation (τ = 13 ± 0.5 ms *versus* 35 ± 1.7 ms at −40 mV). Most notably, hKvβ1.3 converted the Kv1.5 outwardly rectifying current voltage relationship to one showing strong inward rectification. These data suggest that Kv channel current diversity may arise from association with alternatively spliced Kv β-subunits. A simplified nomenclature for the K^+ channel β-subunit subfamilies is suggested.

Voltage-gated K^+ channels (Kv)[1] are important regulators of membrane action potentials as well as many other cellular functions including maintenance of the resting membrane potential, regulating neuron firing, and secretion (1–3). Most tissues contain multiple channel types belonging to one or more Kv gene subfamilies (3–15). However, assigning specific K^+ channel clones to a native current often is difficult since most heterologously expressed Kv channels display either a fast inactivating or a delayed rectifier type current, often with similar pharmacology. While possible factors contributing to this diversity may include Kv channel glycosylation, phosphorylation, and heterotetrameric α-subunit formation within a gene subfamily (16–19), recent studies have shown that β-subunits associate with and functionally alter Kv channel clones in heterologous systems (20–26).

At present, four Kv β-subunits have been reported. Three distinct K^+ channel β-subunits were cloned from rat brain (21, 27). The rat Kvβ1 subunit confers rapid A-type inactivation on the Kv1.1 delayed rectifier channel, while the rat Kvβ2 isoform does not alter K^+ channel current phenotypes in the *Xenopus* oocyte expression system (21). Heinemann and co-workers (27) have reported a third β-subunit from rat brain originally named Kvβ3 that shares 68% identity with rKvβ1 and induces partial inactivation in channels of the Kv1 family. The fourth distinct β-subunit clone, also termed Kvβ3, was isolated from human and ferret heart (23, 24, 26). Identity of this Kvβ3 to previously cloned β-subunits is greatest in the carboxyl-terminal region with complete identity of hKvβ3 and rKvβ1 in the carboxyl 329 amino acids and 85% identity to rKvβ2. However, the first 79 amino acids of hKvβ3 share only ~25% identity with rKvβ1 and do not align with rKvβ2. Fast inactivation of Kv1.4 was accelerated when expressed with hKvβ3 (23–25) and fast inactivation and a 20-mV hyperpolarizing shift in the activation curve was conferred on the delayed rectifier Kv1.5 (23, 26). Human Kvβ3 has no functional effect on the Kv1.1, Kv1.2, and Kv2.1 channel clones (23), although these channels have been postulated to associate with accessory subunits (28, 29).

The complete amino acid identity between rKvβ1 and hKvβ3 in the carboxyl terminus suggests that Kv channel β-subunit isoforms are encoded by a single β-subunit gene. Alternative splicing was suggested previously (21, 26, 30) since the point of divergence between rKvβ1 and hKvβ3 cDNA contained a potential splice junction and the nucleotide sequence identity in the carboxyl terminus was >90%. Calcium channel β-subunits are encoded by four different genes with alternative splicing of the β1 and β2 genes giving rise to multiple β-subunits within these subfamilies (31, 32). Cell-specific alternative splicing of Kv β-subunits may be one mechanism responsible for the diversity of Kv channel current among cell types.

We report here the cloning and characterization of a cDNA from human heart that encodes a unique K^+ channel β-subunit designated hKvβ1.3. The hKvβ1.3 subunit uniquely alters the functional properties of hKv1.5, converting it from a delayed rectifier to a channel with rapid, but partial, inactivation. In addition, this current activates at lower voltages, rectifies at depolarized potentials, and has slowed deactivation. Nucleotide sequence comparison of cDNA and genomic DNA encoding human Kvβ1.3, Kvβ3, and Kvβ1 indicate that these subunits are encoded by a common β-subunit gene, here designated the Kvβ1 subfamily gene. We suggest that the nomenclature be changed to reflect that the Kvβ1 subunit family members are generated through alternative mRNA splicing (Table I).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L47665.

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[1] The abbreviations used are: Kv, voltage-gated K^+ channels; PCR, polymerase chain reaction; kb, kilobase(s).

TABLE I

A simplified nomenclature for a family of vertebrate voltage-gated K^+ channel β-subunit genes

All cloned β-subunits fall into three subfamily categories. The largest is the Kvβ1 subfamily which presently consists of three members. The Kvβ2 and the Kvβ3 subfamilies contain only one member to date.

Subfamily	Proposed	Original	Species/tissue	Chromosomal localization	Reference
Kvβ1 subfamily				h3	26
	Kvβ1.1	Kvβ1	Rat/brain		21
			Human/brain		30
	Kvβ1.2	Kvβ3	Human/ventricle		26
			Human/atrium		23
			Ferret/heart		24
	Kvβ1.3		Human/ventricle		Current communication
Kvβ2 subfamily				h1[a]	—[b]
	Kvβ2.1	Kvβ2	Rat/brain		21
			Bovine/brain		22
			Human/brain		30
Kvβ3 subfamily				Not determined	
	Kvβ3.1	Kvβ3	Rat/brain		27

[a] Preliminary results using human/rodent cell hybrid mapping panel.
[b] S. K. England, V. N. Uebele, J. Kodali, P. B. Bennett, and M. M. Tamkun, unpublished results.

EXPERIMENTAL PROCEDURES

Isolation and Characterization of Kvβ1.3—PCR-generated cDNA fragments corresponding to nucleotides 435 to 1089 of rKvβ2.1 were used to screen 3.5×10^5 amplified recombinants from a λgt10 cardiomyopathic human heart ventricular cDNA library using previously described conditions (33). The primary screening yielded a partial clone (8-82, ~3.0 kb) which was subcloned into pBluescript (KS+) via *Not*I and sequenced using double-stranded templates and appropriate oligonucleotide primers (Sequenase 2.0, United States Biochemical Corp.). This clone lacked an in-frame stop codon 5′ to the first ATG, suggesting it did not represent a full-length coding sequence. Repeated efforts to isolate additional 5′ sequence from a cDNA library were unsuccessful. To clone the 5′ end of 8-82, PCR-generated 260-nucleotide fragments unique to the 5′ end of 8-82 were used to screen 4.2×10^5 amplified recombinants from a λEMBL-3 human genomic library (Clontech). The primary screening yielded one clone that was isolated using Wizard Magic Lambda Preps per the manufacturer's instructions (Promega). This ~14-kb clone was digested by *Sac*I/*Eco*RI, electrophoresed, transferred to nitrocellulose, and hybridized at high stringency (4). A ~4-kb fragment that hybridized to the 260-nucleotide probe was subcloned into pGEM and sequenced. This genomic fragment contained the 26 coding nucleotides missing from the 5′ end of 8-82 and contained in-frame stop codons 5′ to the ATG. The clone was assembled by linearizing clone 8-82 in pBluescript (KS) with *Bgl*II and ligating in a 43-bp fragment containing the 5′ end of 8-82. The completed clone was verified by sequencing and referred to as hKvβ1.3.

Genomic Isolation of hKvβ1.2 and cDNA Isolation of hKvβ1.1—PCR-generated cDNA fragments of Kvβ1.2 (nucleotides −74 to 348) were used to screen 4.2×10^5 amplified recombinants from a λEMBL-3 human genomic library (Clontech). The primary screening yielded an ~13-kb clone that was isolated as described above. This clone was digested with *Sau*3AI, and fragments of multiple sizes were ligated into the *Bam*HI site of pGEM. A plasmid containing a 1-kb genomic fragment positive for Kvβ1.2 was selected by colony hybridization and sequenced using appropriate oligonucleotide primers.

Isolation of hKvβ1.1 cDNA was completed by generating PCR fragments corresponding to nucleotides 1–216 of rKvβ1 and screening 2.8×10^5 unamplified recombinants from a newly constructed λZAPII (Stratagene) cDNA library made from human cerebral cortex mRNA (Clontech). A 4-kb clone was isolated, and plaque-purified clones were recovered by *in vivo* excision yielding hKvβ1.1 in pBluescript (SK−). Nucleotide sequence in various regions was determined as described above.

Electrophysiological Recording and Data Analysis—Templates for *in vitro* cRNA synthesis were prepared by isolating a *Xba*I/*Acc*I fragment of hKvβ1.3 (nucleotides −20 to 1520) from pBluescript (KS), blunting the DNA ends with Klenow and ligating into the *Sma*I site of the modified pSP64T vector (34). This construct was linearized with *Eco*RI prior to cRNA synthesis. The hKv1.5 cRNA template was prepared as described previously (35). Human Kvβ1.3 and hKv1.5 cRNAs were synthesized using the SP6 mMessage mMachine™ kit (Ambion) according to the manufacturer's instructions.

Defolliculated *Xenopus* oocytes were prepared as described previously (19) and injected with approximately 40 nl (4–20 ng) of *in vitro* transcribed cRNA. These dilutions resulted in peak currents of 1–10 μA. Electrophysiological recordings have been described in detail previously (19, 26). Oocytes were bathed in an extracellular solution containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes (pH 7.5 with NaOH). Membrane currents were recorded using a two-microelectrode voltage clamp amplifier from Warner Instruments (New Haven, CT). Values are expressed as mean ± S.E. unless indicated otherwise. All experiments were performed at room temperature.

RESULTS AND DISCUSSION

Cloning and Sequence Analysis—While one potential factor underlying functional diversity of the Kv channels in both brain and heart has been attributed to heteromultimeric formation of various α-subunits (16–19), other possibilities include association of one or more function-altering β-subunits. β-Subunits have been shown to modulate inactivation kinetics, voltage dependence, and current amplitudes of voltage-gated K^+, Na^+, and Ca^{2+} channels (20, 21, 24–26, 32, 36–41), and the voltage and calcium sensitivity of the Ca^{2+}-activated K^+ channels (42). In order to understand the relationship between Kv cardiac clones and native currents, it is necessary to identify the possible α- and β-subunit interactions.

Screening an amplified human heart cDNA library at low stringency with a PCR-generated cDNA probe corresponding to nucleotides 435-1089 of rKvβ2.1 yielded two partial cDNA clones as determined by nucleotide sequencing. The deduced amino acid sequence of one of these clones (hβ2-1) was found to be nearly identical with rKvβ2.1 and likely represents the human homologue of this previously cloned rat subunit (21). The other cDNA (hβ8-82) was most similar to rKvβ1.1 but exhibited little amino acid identity within the postulated amino terminus and lacked a likely translation start site. Additional screens of cDNA libraries did not yield a full-length clone. Screening a human genomic library produced the 26 nucleotides missing from the 5′ end and an obvious translation start site. The initiating methionine was assigned because it represents the first in-frame ATG positioned 3′ of termination codons in an open reading frame encoding a 419-amino acid protein (47 kDa). Hydropathy analysis did not reveal a hydrophobic domain, suggesting that similar to other β-subunits, hKvβ1.3 is likely a cytoplasmic protein. An amino acid se-

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hKvß1.3  MLAARTGAAG SQISEENTKL RRQSGFSVAG KDKSPKKASE NAKDSSLSPS      50
hKvß1.2               MHLYKPACA DIP-PKLGLP -SSESALKCR WHLAVTKTQP      39
rKvß1.1                     MQ VSIACTEHNL -SRNGEDRLL SKQS-TAPNV      32

hKvß1.3  GESQLRARQL ALLREVEMNW YLKLCDLSSE HTTVCTTGMP HRNLGKSGLR     100
hKvß1.2  QAACKPV-PS GAAEQKYVEK F-RVHGI-LQ E--RAE---A Y---------      89
rKvß1.1  VNAARAKFRT VAIIARSLGT FTPQHHI-LK ES-AKQ---K Y---------      82
rKvß2.1    MYPESTTG SPA-LSLRQT GSPGMIY-TR YGSPKRQLQF Y---------      48

         VSCLGLGTWV TFGGQISDEV AERLMTIAYE SGVNLFDTAE VYAAGKAEVI     150
         ---------- ---------- ---------- ---------- ----------     139
         ---------- ---------- ---------- ---------- ----------     132
         ---------- ------T--M --H---L--D N-I------- ---------V      98

         LGSIIKKKGW RRSSLVITTK LYWGGKAETE RGLSRKHIIE GLKGSLQRLQ     200
         ---------- ---------- ---------- ---------- ----------     189
         ---------- ---------- ---------- ---------- ----------     182
         --N------- ---------- IF-------- ---------- ---A--E---     148

         LEYVDVVFAN RPDSNTPMEE IVRAMTHVIN QGMAMYWGTS RWSAMEIMEA     250
         ---------- ---------- ---------- ---------- ----------     239
         ---------- ---------- ---------- ---------- ----------     232
         ---------- ---P------ T--------- ---------- ---S------     198

         YSVARQFNMI PPVCEQAEYH LFQREKVEVQ LPELYHKIGV GAMTWSPLAC     300
         ---------- ---------- ---------- ---------- ----------     289
         ---------- ---------- ---------- ---------- ----------     282
         --------L- --I------- M--------- ----F----- ----------     248

         GIISGKYGNG VPESSRASLK CYQWLKERIV SEEGRKQQNK LKDLSPIAER     350
         ---------- ---------- ---------- ---------- ----------     339
         ---------- ---------- ---------- ---------- ----------     332
         -V-----DS- I-PY------ G-----DK-L -----R--A- --E-QA----     298

         LGCTLPQLAV AWCLRNEGVS SVLLGSSTPE QLIENLGAIQ VLPKMTSHVV     400
         ---------- ---------- ---------- ---------- ----------     389
         ---------- ---------- ---------- ---------- ----------     382
         ---------I ---------- -----A-NA- --M--I---- ----LS-SI-     348

         NEIDNILRNK PYSKKDYRS                                      419
         ---------- ---------                                      408
         ---------- ---------                                      401
         H---S--G-- ---------                                      367
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FIG. 1. **Comparison of hKvβ1.3, hKvβ1.2, rKvβ1.1, and rKvβ2.1 amino acid sequences.** Identical amino acid residues are indicated by *dashes*. The rKvβ1.1, hKvβ1.2, and rKvβ2.1 sequences are from Refs. 21, 23 and 26.

quence comparison between hKvβ1.3, hKvβ1.2, rKvβ1.1, and rKvβ2.1 is illustrated in Fig. 1. The carboxyl-terminal 328 amino acids of hKvβ1.3 are 100% identical with rKvβ1.1 and hKvβ1.2 and share 85% identity with rKvβ$_2$. However, the first 91 amino acids of hKvβ1.3 share <10% identity with hKvβ1.2, rKvβ1.1, and rKvβ2.1.

To determine whether hKvβ1.3 and hKvβ1.2 represent splice variants of the same gene, 3′-untranslated regions were compared. Alignments of this 1800-base pair region showed 99% nucleotide identity between clones isolated from separate individuals, suggesting that hKvβ1.3 and hKvβ1.2 represent splice variants with minor allelic differences. To confirm that Kvβ1.1 is also derived from this gene, Kvβ1.1 was cloned from a human cerebral cortex cDNA library. Three different regions from the 3′-untranslated region corresponding to ~250, ~750, and ~1500 bp 3′ of the translation stop codon were sequenced and showed complete identity with Kvβ1.2 and Kvβ1.3 in this 3′-untranslated region suggesting that all three β-subunits are encoded by this gene. To confirm the splice junction, we attempted to clone the entire Kvβ1 gene from a human genomic library by screening with a probe corresponding to the carboxyl-terminal 328 amino acids of Kvβ1.1. Although several clones were 10–20 kb in length, a full-length gene was not isolated based on the finding that no single clone hybridized to either hKvβ1.2 or hKvβ1.3 amino-terminal specific probes. Likewise, when the unique amino termini of hKvβ1.3 and hKvβ1.2 were used to isolate additional genomic clones, these clones did not hybridize to the carboxyl-terminal probe. The complete gene encoding the hKvβ1 subfamily likely exceeds 40 kb.

Fig. 2 illustrates the genomic sequences of Kvβ1.2 and Kvβ1.3 surrounding the predicted splice site. Both Kvβ1.2 and Kvβ1.3 genomic sequences correspond to their respective cDNA in the region marked exon. Both genomic clones contain a

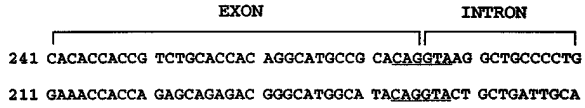

FIG. 2. **Comparison of hKvβ1.3 and hKvβ1.2 genomic sequences surrounding the proposed splice junction.** Genomic sequences corresponding to the variable amino termini cDNA sequences of hKvβ1.3 and hKvβ1.2 are shown. Genomic and cDNA sequences match in the region marked exon and diverge at the exon/intron border. Consensus splice site sequences are indicated by the *underlining* (43).

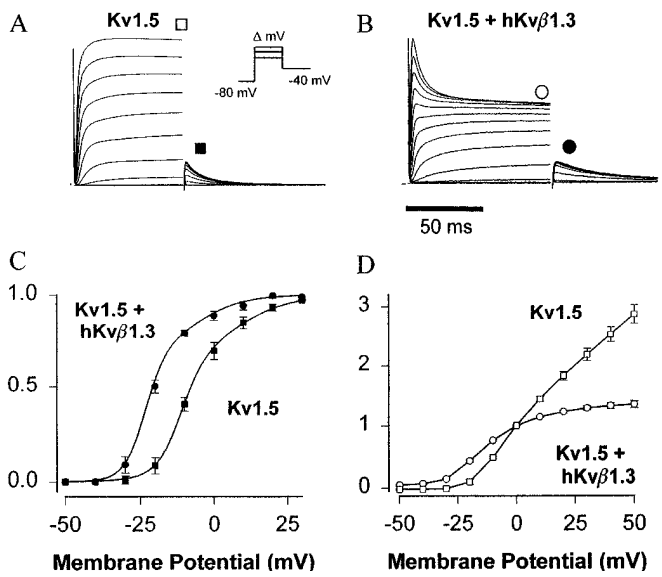

FIG. 3. **Functional effects of hKvβ1.3 on hKv1.5.** Whole cell potassium current was recorded from *Xenopus* oocytes expressing hKv1.5 in the absence (*A*) and presence (*B*) of hKvβ1.3, and each cell was normalized to peak current at +50 mV (= 1). The cells were voltage-clamped at a holding potential of −80 mV for 30 s prior to a variable test potential (shown as inset voltage protocol) and then stepped to −40 mV to record deactivating tail currents. In *A*, the test step was 75 ms in duration which allowed steady state current levels to be attained at each potential. This duration was increased to 100 ms in the presence of the hKvβ1.3 subunit (*B*) to permit steady state to be achieved. Normalized tail currents of hKv1.5 in the presence (*closed circles*) and the absence (*closed squares*) of hKvβ1.3 are plotted as a function of test step potential (*C*). *D* represents the steady-state current-voltage relationship for Kv1.5 alone (*open squares*) and Kv1.5 coexpressed with hKvβ1.3 (*open circles*). Potassium current was measured at steady state (see *open symbols* in *A* and *B*) and plotted as a function of the test potential. In order to compare different cells, the current was normalized by dividing the current at each membrane potential by the value measured at 0 mV (= 1). Note that hKvβ1.3 causes an apparent rectification and that the channels begin to open at more negative membrane potentials compared to Kv1.5 alone. *Symbols* represent between 5 and 10 observations and are plotted as the mean ± 2 × S.E.

consensus sequence for donor/acceptor splice sites as shown by the underlined sequence (43). These data provide further evidence that at least three β-subunits result from alternative splicing. Therefore, differential regulation of Kv β-subunit expression and alternative splicing are likely to be two mechanisms regulating Kv channel diversity. Further *in situ* analysis and antibody-based immunohistochemical localization of the Kv β-subunits will further our understanding of Kv channel α- and β-subunit association.

Functional Expression of hKvβ1.3—Fig. 3 illustrates the effects of Kvβ1.3 on hKv1.5 currents. Current tracings were obtained during voltage clamp steps to depolarizing membrane potentials where outward current is activated and tail currents are measured during channel deactivation upon steps to −40 mV. Human Kv1.5 normally displays a modest degree of outward rectification and begins to activate at a membrane potential of about −30 mV (Fig. 3*A*) (44). In the presence of Kvβ1.3,

Kv1.5 current displays a time-dependent decay or partial inactivation at large depolarizing steps (Fig. 3*B*) that occurs only at membrane potentials greater than approximately 0 mV. Fig. 3*B* illustrates also the slower rate of channel deactivation seen in the presence of Kvβ1.3 relative to the Kv1.5 alone. These deactivating tail currents were best fit by one exponential with time constants of 13.2 ± 0.5 ms for wild-type Kv1.5 and time constants of 34.9 ± 1.7 ms in the presence of Kvβ1.3 at −40 mV ($p < 0.05$). Due to the effect of the hKvβ1.3 which decreases current at larger membrane potentials, the magnitude of the tails may be underestimated. Time constants for the apparent inactivation induced by Kvβ1.3 were 8.9 ± 0.3 ms at +50 mV and 9.2 ± 0.45 ms at +30 mV ($n = 12$) ($p > 0.1$, not significant). Fits of the decay at less positive potentials were less reliable and were not done.

An additional effect observed during co-expression of Kv1.5 with Kvβ1.3 was that the threshold for Kv1.5 channel activation occurred at more negative potentials. The shift in the activation curve toward more negative potentials is illustrated in Fig. 3*C* where the amplitude of the tail currents is plotted as a function of the membrane potential. Since the driving force is constant during this measurement, the curve reflects the fraction of channels open at each membrane potential. The average midpoint of the activation curve for the hKv1.5 was −7.1 ± 0.5 mV ($n = 6$) whereas in the presence of the hKvβ1.3 it was −20 ± 0.5 mV ($n = 6$). Fig. 3*D* shows steady state outward current measured during depolarizing steps. Note that hKv1.5 current is observed at more negative potentials when hKvβ1.3 is present, even at potentials that do not show apparent inactivation (*i.e.* −20 mV). At membrane potentials greater than approximately 0 mV, hKv1.5 current in the presence of hKvβ1.3 is suppressed relative to the Kv1.5 alone, thereby converting this apparent outwardly rectifying current voltage relationship to one that shows inward rectification. Future detailed analysis of these interactions will elucidate the underlying effects of hKvβ1.3.

CONCLUSIONS

Discovery of the novel Kvβ1.3 subunit and that alternative mRNA splicing generates multiple function altering β-subunits further complicates determination of the relationship between cardiac clones and native currents. Future cell-specific localization and co-purification studies using Kv β-antibodies will enable us to understand both the pattern of Kv β-subunit isoform expression and the Kv channels with which these subunits associate. In addition, analysis of the mechanisms by which Kvβ1.3 alters the voltage sensitivity, inactivation, and rectification of Kv1.5 will advance our understanding of Kv channel function.

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REFERENCES

1. Rudy, B. (1988) *Neuroscience* **25,** 729–749
2. Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 2nd Ed, Sinauer Associates Inc., Sunderland, MA
3. Sakmann, B., and Trube, G. (1984) *J. Physiol. (Lond.)* **347,** 641–657
4. Roberds, S. L., and Tamkun, M. M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 1798–1802
5. Chandy, K. G., and Gutman, G. A. (1995) in *Handbook of Receptors and Channels: Ligand- and Voltage-gated Ion Channels* (North, R. A., ed) pp. 1–71, Boca Raton, FL
6. Hume, J. R., and Uehara, A. (1985) *J. Physiol. (Lond.)* **368,** 525–544
7. Heidbuchel, H., Vereecke, J., and Carmeliet, E. E. (1990) *Circ. Res.* **66,** 1277–1286
8. Sanguinetti, M. C., and Jurkiewicz, N. K. (1990) *J. Gen. Physiol.* **96,** 195–215
9. Sanguinetti, M. C., and Jurkiewicz, N. K. (1991) *Am. J. Physiol.* **260,** H393–H399
10. Balser, J. R., Bennett, P. B., and Roden, D. (1990) *J. Gen. Physiol.* **96,** 835–863
11. Yue, D. T., and Marban, E. (1988) *Pfluegers Arch.* **413,** 127–133
12. Boyle, W. A., and Nerbonne, J. M. (1991) *Am. J. Physiol.* **260,** H1236–H1247
13. Benndorf, K., Markwardt, F., and Nilius, B. (1987) *Pfluegers Arch.* **413,** 127–133
14. Tseng, G. N., and Hoffman, B. F. (1989) *Circ. Res.* **64,** 633–647
15. Escande, D., Coulombe, A., and Faivre, J. (1985) *Am. J. Physiol.* **252,** H142–H148
16. Ruppersberg, J. P., Schroter, K. H., Sakmann, B., Stocker, M., Sewing, S., and Pongs, O. (1990) *Nature* **345,** 535–537
17. Christie, M. J., North, R. A., Osborne, P. B., Douglass, J., and Adelman, J. P. (1990) *Neuron* **4,** 405–411
18. Isacoff, E. Y., Jan, Y. N., and Jan, L. Y. (1990) *Nature* **345,** 530–534
19. Po, S. S., Roberds, S. L., Snyders, D. J., Tamkun, M. M., and Bennett, P. B. (1993) *Circ. Res.* **72,** 1326–1336
20. Chouinard, S. W., Wilson, G. F., Schlimgen, A. K., and Ganetzky, B. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6763–6767
21. Rettig, J., Heinemann, S. H., Wunder, F., Lorra, C., Parcej, D. N., Dolly, J. O., and Pongs, O. (1994) *Nature* **369,** 289–294
22. Scott, V. E., Rettig, J., Parcej, D. N., Keen, J. N., Findlay, J. B., Pongs, O., and Dolly, J. O. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1637–1641
23. Majumder, K., De Biasi, M., Wang, Z., and Wible, B. A. (1995) *FEBS Lett.* **361,** 13–16
24. Morales, M. J., Castellino, R. C., Crews, A. L., Rasmusson, R. L., and Strauss, H. C. (1995) *J. Biol. Chem.* **270,** 6272–6277
25. Castellino, R. C., Morales, M. J., Strauss, H. C., and Rasmussen, R. L. (1995) *Am. J. Physiol.* **38,** H385–H391
26. England, S. K., Uebele, V. N., Shear, H., Kodali, J., Bennett, P. B., and Tamkun, M. M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6309–6313
27. Heinemann, S. H., Rettig, J., and Pongs, O. (1995) *Biophys. J.* **68,** A361
28. Rehm, H., and Lazdunski, M. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 4919–4923
29. Trimmer, J. S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10764–10768
30. McCormack, K., McCormack, T., Tanouye, M., Rudy, B., and Stuhmer, W. (1995) *FEBS Lett.* **370,** 32–36
31. Powers, P. A., Liu, S., Hogan, K., and Gregg, R. G. (1992) *J. Biol. Chem.* **267,** 22967–22972
32. Isom, L. L., De Jongh, K. S., and Catterall, W. A. (1994) *Neuron* **12,** 1183–1194
33. Tamkun, M. M., Knoth, K., Walbridge, J. A., Kroemer, H., Roden, D., and Glover, D. (1991) *FASEB J.* **5,** 331–337
34. White, M. M., Chen, L., Kleinfield, R., Kallen, R. G., and Barchi, R. L. (1991) *Mol. Pharmacol.* **39,** 604–608
35. Po, S. S., Snyders, D. J., Baker, R., Tamkun, M. M., and Bennett, P. B. (1992) *Circ. Res* **71,** 732–736
36. De Waard, M., Pragnell, M., and Campbell, K. P. (1994) *Neuron* **13,** 495–503
37. Messner, D. J., Feller, D. J., Scheuer, T., and Catterall, W. A. (1986) *J. Biol. Chem.* **261,** 14882–14890
38. Messner, D. J., and Catterall, W. A. (1986) *J. Biol. Chem.* **261,** 14882–14890
39. McHugh-Sutkowski, E., and Catterall, W. A. (1990) *J. Biol. Chem.* **265,** 12393–12399
40. Makita, N., Bennett, P. B., and George, A. L. (1994) *J. Biol. Chem.* **269,** 7571–7578
41. Bennett, P. B., Makita, N., and George, A. L. (1993) *FEBS Lett.* **326,** 21–24
42. McManus, O. B., Helms, L. M. H., Pallanck, L., Ganetzky, B., Swanson, R., and Leonard, R. J. (1995) *Neuron* **14,** 645–650
43. Mount, S. M. (1982) *Nucleic Acids Res.* **10,** 459–472
44. Snyders, D. J., Tamkun, M. M., and Bennett, P. B. (1993) *J. Gen. Physiol.* **101,** 513–543