Separable Kvβ Subunit Domains Alter Expression and Gating of Potassium Channels*

(Received for publication, May 21, 1997, and in revised form, July 21, 1997)

Eric A. Accili‡§, Johann Kiehn‡§, Qing Yang‡§, Zhiguo Wang‡§, Arthur M. Brown§, and Barbara A. Wible**

From the Rammelkamp Center for Research, MetroHealth Campus, and the Departments of Physiology and Biophysics and Biochemistry, Case Western Reserve University, Cleveland, Ohio 44109-1998

Kvβ subunits have been shown to affect kinetic properties of voltage-gated K⁺ channel Kvα subunits and increase the number of cell surface dendra toxin-binding sites when coexpressed with Kv1.2. Here, we show that Kvβ2 alters both current expression and gating of Kvα channels and that each effect is mediated by a distinct Kvβ2 domain. The Kvβ1.2 N terminus or Kvβ1-blocking domain introduced steady state current block, an apparent negative shift in steady state activation, and a slowing of deactivation along with a dramatic reduction in single channel open probability. N-terminal deletions of Kvβ1.2 no longer altered channel kinetics but promoted dramatic increases in Kv1.2 current. The conserved Kvβ1 C terminus or Kvα expression domain alone was sufficient to increase the number of functional channels. The same effect was observed with the normally noninactivating subunit, Kvβ2. By contrast, Kvβ1.5 currents were reduced when coexpressed with either the Kvβ1 C terminus or Kvβ2, indicating that the Kvα1 expression domain has Kvα1 isoform-specific effects. Our results demonstrate that Kvβ subunits consist of two domains that are separable on the basis of both primary structure and functional modulation of voltage-gated K⁺ channels.

Voltage-gated K⁺ channels consist of pore-forming α-subunits (Kvα) complexed with modulatory Kvβ subunits. For channels in the Kv1 subfamily, a number of related Kvβ subunits have been cloned and shown to specifically modulate currents generated by Kvα1 subunits in heterologous expression systems. A variety of Kvβ subunits have been cloned from brain (1–3) and heart (4–7). Based on sequence homology, they have been classified into three subfamilies: Kvβ1, Kvβ2, and Kvβ3. All Kvβ subunits are predicted to be cytoplasmic proteins and can be separated into two domains: N terminus and C terminus. The homology among subfamilies resides in the C terminus, which constitutes the majority of the protein and is responsible for binding to the Kvα1 N terminus (8–10). The Kvβ1 subfamily currently consists of three alternatively spliced isoforms: Kvβ1.1 (2), Kvβ1.2 (4–6), and Kvβ1.3 (7, 9). These subunits share conserved C-terminal domains of 329 amino acids attached to unique N-terminal domains ranging from 72 to 91 residues.

Kvβ subunits have dual effects on Kvα1 subunit currents. The three Kvβ1 isoforms, as well as Kvβ3, introduce inactivation into Kvα1 subunit currents but with variable potency (2, 3, 9). The Kvβ1 N termini are thought to produce inactivation by acting like a ball peptide to mimic N-type inactivation (2, 11). Coexpression of Kvβ1.2 with Kv1.5 has also been shown to shift the activation curve to more negative voltages and to increase the time constant of deactivation (6), although these effects may be secondary to open channel block (11). In contrast, Kvβ2 subunits do not alter the inactivation or deactivation properties of coexpressed Kvα1 subunits but do shift activation kinetics of Kv1.5 to more negative voltages (2, 12).

More recently, Kvβ subunits were shown to increase the surface expression of Kv1.2 as measured by an increase in dendrotoxin-binding sites at the plasma membrane of transfected cells (13). The functional consequences of this increase as well as the Kvβ subunit domain responsible for this effect have not been determined. The Drosophila Kvβ subunit encoded by the hyperkinetic locus has also been shown to alter both the kinetics and current amplitude of Shaker Kvα subunits (14), but it is not known whether the increase results from alterations in intrinsic inactivation or functional expression. Interestingly, a recent report found that Kvβ2 did not increase the surface expression of Shaker channels, although the two proteins were found to associate in the endoplasmic reticulum (15).

We have examined the functional consequences of Kvβ1 assembly with Kvα1 channels and determined the Kvβ1 domains responsible. We show that the Kvβ1.2 C terminus (Kvβ1-C) as well as Kvβ2 produce dramatic increases in Kv1.2 currents when coexpressed in Xenopus oocytes. This increase in functional expression is due to an increase in the number of functional channels and not by alterations in single channel properties. In contrast, Kvβ1.5 currents show a dramatic decrease upon coexpression with Kvβ1-C, indicating that the nature of the expression effect is Kvα1 isoform-dependent. All kinetic effects of Kvβ1.2 can be explained by open channel block of Kv1.2 by the Kvβ N terminus. Thus, our studies of noninactivating Kvα1 channels have demonstrated that Kvβ1.2 consists of two functional domains: a Kvα1-blocking domain located in the first 20 amino acids of the N terminus and a Kvα1 expression domain consisting of the entire C terminus.

** Experimental Procedures

Yeast Two-hybrid Interaction—The Kv1.2 cDNA was a gift from O. Pongs. HERG cDNA was kindly provided by M. Keating. Kvα1.2 was cloned as described previously (4). Kvβ2 was obtained by reverse tran-
Data Analysis Single Channels—Data were low pass filtered at 2 kHz (-3db, 4-pole Bessel filter) before digitalization at 10 kHz. Pclamp software (Axon Instruments) was used for generation of the voltage-pulse protocols and for data acquisition. All single channel measurements were leak subtracted in Fetchan and analyzed using TRANSIT software (17). This produced histograms for the open time, ensemble current traces, and values of open probability. The maximum likelihood method is utilized by TRANSIT to determine time constants from open time data for open time from TRANSIT and presented on a log scale as described previously (17). Single channel parameters were given as the means ± S.D.

After forming a giga-seal, single channel I-V protocols were performed to ensure that the investigated channel had the expected activ-
Functional Domains of Kvβ Subunits

Fig. 2. Deletion of 20 N-terminal Kvβ1.2 residues unmasks a dramatic increase in Kv1.2 currents. Whole cell currents measured in Xenopus oocytes injected with Kv1.2 cRNA (2 ng/μl) alone (A) or coinjected with Kvβ1.2 (100 ng/μl) (B) or Kvβ1.2-NΔ20 (100 ng/μl) (C). Oocytes were held at −80 and pulsed to +80 in 10 mV steps of 100 ms duration. D, current amplitudes were measured at the end of the step and displayed in a bar plot showing whole cell current levels measured in groups described in A–C. Values represent the means ± S.E., and ** indicates a significant difference from the value for Kv1.2 + Kvβ1.2-NΔ20 currents (p < 0.05, one-way analysis of variance, n = 10 oocytes for each group).

Fig. 3. Kvβ1 C terminus and Kvβ2 increase functional expression of Kv1.2 in Xenopus oocytes. A, cRNAs for Kv1.2 (2 ng/μl) and Kvβ1 C terminus (250 ng/μl) or Kvβ2 (250 ng/μl) were injected into Xenopus oocytes. Current amplitudes were measured at the end of the pulse to +80 mV. Values represent the means ± S.E., and ** indicates a significant difference from the values for Kv1.2 + Kvβ1-C and Kv1.2 + Kvβ2 (p < 0.05, one-way analysis of variance, n = 21–29 oocytes for each group in total in two injection series).

The Kv1.2 C terminus did not alter the inactivation properties of Kv1.2 (not shown) but did result in a significant increase (about 5-fold) in the amplitude of Kv1.2 currents when cRNAs were coinjected in Xenopus oocytes (Fig. 3). Yeast two-hybrid experiments showing the binding of Kvβ1-C to the N terminus of Kv1.2 strongly suggest that the dramatic increase in functional surface expression of this channel is due to the direct interaction of these subunits. Coexpression of Kv1.2 with either Kvβ1.2-NΔ20 or Kvβ1-C increased current amplitudes to similar extents, by factors of 4.19 and 4.18, respectively (n = 4–5 oocytes in one injection series). This indicates that the Kv01 expression domain consists of the Kvβ C terminus alone. Coinjection of saturating amounts of a normally noninactivating Kvβ subunit, Kvβ2, produced similar results (Fig. 3). The high degree of homology between the C-terminal domains of Kvβ1 and Kvβ2 subunits is reflected in the similar amplitude increases elicited by the two subunits.

The increase in current amplitude occurred in both low K+ (5 mM) and high K+ (50 mM) solutions ruling out external K+ as the major cause of the increase in the maximal conductance. As a test for specificity, we coexpressed Kvβ1.2 or Kvβ1.2-NΔ20 with HERG, the K+ channel responsible for the rapid component of the delayed rectifier K+ current in human heart. In two injection series, we found no significant changes in kinetics or current amplitude when HERG was coexpressed with Kvβ1.2 or Kvβ1.2-NΔ20 (data not shown).

Single Channel Measurements Demonstrate That the Increase in Kv1.2 Current Mediated by the Kvβ1 C Terminus Is Due to an Increase in the Number of Functional Channels at the Cell Surface.—To determine the mechanism by which the Kvβ1 C terminus increased functional expression, we compared the single channel properties of Kv1.2 alone and in the presence of Kvβ1.2 or the noninactivating Kvβ1.2-NΔ20. In a previous report, we demonstrated that the properties of single Kv1.2 channels are altered by Kvβ1.2 (9). These were confirmed here at a test potential of +70 mV and compared with single channel measurements of Kv1.2 and Kvβ1.2-NΔ20 to see whether the mutant Kvβ altered single channel properties. Kv1.2 cRNA injected alone produced single channels with openings in the outward direction, short closures, and a mean amplitude of 1.49 ± 0.10 pA (n = 7). The channels were open and showed no apparent inactivation (Fig. 4A). The open time distribution was fitted with a single exponential to give an open time of 4.1 ms (Fig. 4D). The open probability (P_o) distribution gave a mean of 0.81 and showed virtually no inactivation as well (Fig. 4G).

Expression of Kv1.2 with Kvβ1.2 resulted in single channels with completely different kinetics as described previously. Channels opened frequently in the beginning of the trace followed by long closures before reopening (Fig. 4B). The amplitude of the openings was reduced to 1.13 ± 0.14 pA (n = 7), presumably because blocking events happened very fast, preventing the channels from reaching full amplitude under our recording conditions. Subsequently, the mean open time de-
creased to 0.59 ms, and the mean open probability was reduced dramatically to 0.05 (Fig. 4, E and H). The mean of eight patches gave the following values: \( t = 0.66 \pm 0.08 \) and \( P_0 = 0.08 \pm 0.02 \).

When Kv1.2 was coexpressed with Kvβ1.2-NΔ20, we observed single channels with similar properties to Kv1.2 alone (Fig. 4, compare C and A). The amplitude was \( 1.52 \pm 0.13 \) (\( n = 6 \)). Also, the open time distribution (\( t = 4.1 \) ms) and mean open probability (0.82) were very similar. For six patches, the values were: \( t = 3.85 \pm 0.30 \) ms and \( P_0 = 0.82 \pm 0.02 \). Because of the similarity in single channel parameters, we conclude that the increase in functional expression of Kv1.2 by Kvβ1.2-NΔ20 must be due to an increase in the number of functional channels.

Open Channel Block of Kv1.2 by Kvβ1.2-N Terminus Alters the Kinetics of Kv1.2 Currents—The deletion experiments indicated that inactivation of Kv1.2 currents by Kvβ1.2 was due to residues within the first 20 amino acids of Kvβ1.2. These 20 amino acids constitute a putative ball in the proposed "ball and chain" model of inactivation of Kvα1 subunits by Kvβ1 subunits (2, 4). Modeling studies have suggested that the open channel blocking action of Kvβ1.2 is responsible for the observed negative shift in steady state activation and the slowing of deactivation of Kv1.5 (11). Thus, removal of the ball from Kvβ1.2 would be expected to eliminate alterations in activation and deactivation as well as inactivation. To test this, activation curves of Kv1.2 were generated in the absence and the presence of inactivating and noninactivating Kvβ subunits (Fig. 5A). In the presence of Kvβ1.2, Kv1.2 currents exhibited a leftward shift of almost 12 mV in the activation curve compared with Kv1.2 expressed alone (Fig. 5A). On the other hand, neither of the noninactivating Kvβ fragments, the Kvβ1 C terminus nor Kvβ2, produced any significant changes in the activation curve of Kv1.2.

Similarly, deactivation of Kv1.2 was significantly slowed in the presence of Kvβ1.2 but neither the Kvβ1 C terminus nor Kvβ2 produced large changes in deactivation compared with Kv1.2 alone (Fig. 5B). Furthermore, neither the Kvβ1 C terminus nor Kvβ2 produced any significant changes in the activation time constants of Kv1.2 (data not shown). Therefore, the kinetic changes in Kv1.2 currents that have been observed in the presence of Kvβ1.2 are due to the presence of a functional ball peptide on the Kvβ N terminus plugging the Kv1.2 pore. When the Kvβ1.2 N terminus is removed or when the normally noninactivating Kvβ2 subunit is tested, the kinetics of Kv1.2 are unaltered even though the current amplitudes are dramatically increased (see Fig. 3). Thus, there is a clear separation in functional effects between the structurally distinct Kvβ1.2 N- and C-terminal domains.

The Maximal Fraction of Kv1.2 Current Blocked by the Kvβ1.2 N Terminus Is Much Larger than Previously Suspected—To quantify the block by the Kvβ1.2 N terminus, we initially considered fractional block or inactivation as the difference between peak current and steady state current divided by the peak current as done previously (2, 9, 11). The fractional block is plotted as a function of Kvβ1.2 concentration in Fig. 6A. A maximal peak to steady state inactivation of 0.66 was determined similar to results reported previously (9). A problem with this method is that it implies that 34% of the total current remained unblocked. This is clearly not consistent with single channel measurements, which show that the open probability was reduced to 0.08 in the presence of Kvβ1.2. We assumed that this inconsistency was due to a reduction in the peak current, masked by the dramatic concomitant increase...
produced by Kvβ1-C, thereby overestimating the amount of sustained outward current. A more accurate measure of the blocking effect of Kvβ1.2 was determined from the steady state currents to eliminate bias of current block due to normalization with the peak current. First, we measured the steady state currents to eliminate bias of current block due to normalization with the peak. Second, we measured the steady state currents at each concentration of RNA were calculated in oocytes injected with 2 ng/μl of Kv1.2 alone or with 250 ng/μl of Kvβ1.2, Kvβ1 C terminus, or Kvβ2 using a high K' NMDG+ solution (see "Experimental Procedures"). Symbols are as in panel A.

The single channel studies support the large reduction in current data, which suggested a maximal block of only 60–70% based on peak to steady state current (2, 9). On the other hand, the value of 0.90 is very close to the value of 0.94 determined from fractional steady state current block (Fig. 6C, shown above). The single channel and macroscopic data confirm that the N-terminal domain of Kvβ1.2 is able to block much more available outward current than previously suspected and significantly reduce the peak current as suggested previously (2, 9). This also implies that even small amounts of an inactivating Kvβ subunit like Kvβ1.2 in channel complexes could result in significant reductions in open probability and outward current.

The Effects of the C-terminal Domain of Kvβ1.2 Differ between Kvo1 Subunits—We have demonstrated that the N- and C-terminal domains of Kvβ1.2 produce distinct effects on Kv1.2. Previous studies have shown that Kvβ1 subunits, by virtue of their N-terminal domains, produce various kinetic alterations in expressed Kv1.5 currents similar to those we found with Kv1.2 (4, 6, 7, 11, 12). We asked whether Kvβ1-C would also enhance Kv1.5 expression as observed with Kv1.2. Surprisingly, Kv1.5 currents were significantly decreased upon coexpression with Kvβ1-C as shown in Fig. 7A, where comparisons were made directly with Kv1.2. In four injection series, average Kv1.5 current levels decreased from 9.31 ± 0.94 pA (n = 31) to 3.24 ± 0.32 pA (n = 31) when expressed with the Kvβ1-C terminus. A decrease in Kv1.5 current levels was also noted upon coexpression with Kvβ2 (Fig. 7B). These data show that the Kvo1 expression domain of Kvβ1 can produce different and even opposite effects on different Kvo1 subunits. This differs from the Kvo1-blocking domain, which effectively blocks
Kvα1 subunit currents, although the potency with which different N-terminal domains introduce block may vary (2, 9).

**DISCUSSION**

Kvβ subunits have multiple effects on voltage-gated channels of the Kv1 subfamily. Our results show that Kvβ1.2 can be divided into two structural and functional domains, one of which, the N terminus, acts as the Kvα1-blocking domain by producing inactivation. Here we have shown that the Kvβ1.2-C terminus alone is able to bind to the Kvα1 subunit and promote the alteration of current expression. Thus, the Kvβ1.2-C terminus acts as the Kvα1 expression domain. Coexpression of N-terminal Kvβ1.2 deletion mutants with Kv1.2 resulted in non-inactivating currents and increased current amplitude (up to 14-fold). Single channel amplitude and open probability were not affected by coexpression of Kv1.2 with the deletion mutant Kvβ1.2-NΔ20. Therefore, the observed increase in whole cell current amplitude was due to an increased number of functional channels in the membrane. An increase in the number of functional Kv1.2 channels must occur because the single channel open probability of Kv1.2 determined here and previously (9) is approximately 0.8, yet the increase in Kv1.2 currents by noninactivating Kvβ1.2 deletions was at least 4-fold. This increase is in agreement with experiments in mammalian cells showing enhanced transport of Kv1.2 channels to the membrane in the presence of Kvβ subunits (13). Yeast two-hybrid interaction data presented here and previously (8, 9) suggest that the increase in current results from a direct interaction between the subunits and have shown that the regions critical for interaction are located in the N terminus of Kvα subunits and the Kvβ subunit C terminus. This is in agreement with coimmunoprecipitation studies indicating that the interaction of Kvβ subunits with Kvα subunits does not require the Kvβ N-terminal domain (19).

Macroscopic and single channel data have shown that the first 20 amino acids of the N terminus of Kvβ1.2 contain the β-ball peptide. This domain introduces current block (inactiva-
Functional Domains of Kvβ Subunits

**Figure 7. Kvβ1 C terminus and Kvβ2 increase functional expression of Kv1.2 but decrease functional expression of Kv1.5.** Bar plots showing increases in Kv1.2 and decreases in Kv1.5 whole cell current levels produced by Kvβ1-C (A) and Kvβ2 (B). Oocytes were held at −80 mV and pulsed to +70 mV for 150 ms. Current amplitudes were measured at the end of the step. Values represent the means ± S.E. * and ** indicate a significant difference from the value of current block produced by Kv1.2 when calculated as a fraction of the peak current. Significant fractional block, as estimated from the ratio of steady state currents, can occur in the absence of any apparent inactivation as estimated from the ratio of steady state to peak current. Hence, the ratio of steady state to peak current is not a reliable indication of current block.

Kvβ2, a noninactivating Kvβ subunit, resembles the Kvβ1 C terminus with respect to functional modifications of Kvα1 currents. As for Kvβ1-C, we observed large increases in current expression and little change in the kinetic parameters of Kv1.2 channels with Kvβ2. Kvβ2 also promoted dramatic decreases in Kv1.5 current expression. In yeast two-hybrid assays, Kvβ2 was also shown to interact with the N termini of Kv1.2 and Kv1.5. These results were not unexpected because there is extensive homology in the C-terminal domains of all Kvβ subunits. A recent report showing that Kvβ2 and Kvβ1 bind to the same region on the Shaker N terminus emphasizes the similarity in the two subunits (20). However, in contrast to the Kvβ1 subfamily, a function for the Kvβ2 N terminus has not been described.

Surprisingly, the C terminus of Kvβ1 produced a significant decrease in Kv1.5 current levels in contrast to the dramatic increase observed with Kv1.2. Thus, the action of the Kvα1 expression domain apparently differs depending upon the particular Kvα1 subunit it is complexed with. The origin of these differences are presently unknown. Surface expression of Kv1.1, Kv1.3, and Kv1.6, in addition to Kv1.2, were all shown to increase upon coexpression with Kvβ2 but to a lesser extent than Kv1.2 (13). In contrast, the rate of maturation of Shaker channels actually decreased when coexpressed with Kvβ2 (15).

In heterologous expression systems, Shaker subunits are fully glycosylated and transferred to the Golgi apparatus in the absence of Kvβ subunits (21) unlike Kv1.2, which is poorly glycosylated and found predominantly in the endoplasmic reticulum (13). Taken together, this would suggest that Kv1.2, Kv1.1, Kv1.3, and Kv1.6 subunits exist predominantly in an immature form in the absence of Kvβ subunits compared with Kv1.5 and Shaker. Kvβ subunits would then promote transit of the former group to the cell surface resulting in increased expression. This hypothesis would not, however, explain the decrease in Kv1.5 expression we observed or the decrease in maturation of Shaker channels in the presence of Kvβ2 (15). The decrease in functional expression of Kv1.5 may be due to a nonspecific competition for factors required for expression between Kv1.5 and Kvβ as suggested for Shaker (15) or may be due to other mechanisms specific to Kv1.5-Kvβ interactions. Quantitative differences in Kvα-Kvβ interactions may also exist that contribute to the functional differences described. For example, Nakahira et al. (19) showed that significantly less Kv1.5 protein was communoprecipitated with anti Kvβ subunit antibodies compared with Kv1.2, possibly because of a lower affinity between Kvβ and Kv1.5 subunits.

In summary, cytoplasmic Kvβ subunits affect Kvα1 channels in two ways through distinct protein domains. The Kvβ C terminus, which is highly conserved among all subfamilies of Kvβ subunits thus far described, acts as a Kvα1 expression domain, and in the case of Kv1.2, increases the number of functional channels at the cell surface. Further experiments will be required to determine the origin of the decrease in Kv1.5 expression mediated by this domain. For Kv1.2, all of the apparent kinetic alterations can be attributed to the variable Kvβ1 N-terminal domains. Because all Kvβ subunits share similar C-terminal regions, it is tempting to speculate that their original association with ion channels served to modulate K+ currents through alteration of expression. Kvβ2, for example, promotes alterations in expression, but the N terminus...
apparently does not alter channel kinetics. Distinct Kvβ isoforms with unique N-terminal domains might then have evolved to fine tune channel function. Together, these considerations suggest that the relative levels of distinct Kvα1 and Kvβ subunits within a cell will have profound consequences on the composition and properties of native K+ channel complexes.

Acknowledgments—We thank Drs. A. E. Lacerda, D. Fedida, G. Kirsch, R. Dumaine, E. Ficker, and M. L. Roy for discussion; Tom Carroll and Dr. W. Q. Dong for technical assistance; and P. Kiehn for help with the manuscript.

REFERENCES
1. Scott, V. E. S., Rettig, J., Parcej, D. N., Keen, J. N., Findlay, J. B. C., Pongs, O., and Dolly, J. O. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1637–1641
2. Rettig, J., Heinemann, S. H., Wunder, F., Lorra, C., Parcej, D. N., Dolly, J. O., and Pongs, O. (1994) Nature 369, 289–294
3. Heinemann, S. H., Rettig, J., Wunder, F., and Pongs, O. (1995) FEBS Lett. 377, 383–389
4. Majumder, K., DeBiasi, M., Wang, Z., and Wible, B. A. (1995) FEBS Lett. 361, 13–16
5. Morales, M. J., Castellino, R. C., Crews, A. L., Rasmusson, H. L., and Strauss, H. C. (1995) J. Biol. Chem. 270, 6272–6277
6. 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
7. England, S. K., Uebele, V. N., Kodali, J., Bennett, P. B., and Tamkun, M. M. (1995) J. Biol. Chem. 270, 28531–28534
8. Yu, W., Xu, J., and Li, M. (1996) Neuron 16, 441–453
9. Wang, Z., Kiehn, J., Yang, Q., Brown, A. M., and Wible, B. A. (1996) J. Biol. Chem. 271, 28311–28317
10. Sewing, S., Rupeper, J., and Pongs, O. (1996) Neuron 16, 455–463
11. DeBiasi, M., Wang, Z., Accili, E. A., Wible, B. A., and Fedida, D. (1997) Am. J. Physiol. 272 (Heart Circ. Physiol. 41), H2932–H2941
12. Heinemann, S. H., Rettig, J., Graack, H. B., and Pongs, O. (1996) J. Physiol. 493.3, 625–633
13. Shi, G., Nakahira, K., Hammond, S., Rhodes, K. J., Schechter, L., and Trimmer, J. S. (1996) Neuron 16, 843–852
14. Chouinard, S. W., Wilson, G. F., Schlimgen, A. K., and Ganetzky, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6763–6767
15. Nagaya, N., and Papazian, D. (1997) J. Biol. Chem. 272, 3022–3027
16. Tagliatela, M., Wible, B. A., Caporaso, R., and Brown, A. M. (1994) Science 264, 844–847
17. VanDongen, A. M. J. (1996) Biophys. J. 70, 1303–1315
18. Kiehn, J., Lacerda, A. E., Wible, B., and Brown, A. M. (1996) Circulation 94, 2572–2579
19. Nakahira, K., Shi, G., Rhodes, K. J., and Trimmer, J. S. (1996) J. Biol. Chem. 271, 7084–7099
20. Xu, J., and Li, M. (1997) J. Biol. Chem. 272, 11728–11735
21. Schulteis, C. T., John, S. A., Huang, Y., Tang, C.-Y., and Papazian, D. M. (1995) Biochemistry 34, 1725–1733