Conserved Kv4 N-terminal Domain Critical for Effects of Kv Channel-interacting Protein 2.2 on Channel Expression and Gating*

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Association of Kv channel-interacting proteins (KChIPs) with Kv4 channels leads to modulation of these A-type potassium channels (An, W. F., Bowlby, M. R., Betty, M., Cao, J., Ling, H. P., Mendoza, G., Hinson, J. W., Mattsson, K. I., Strasse, B. W., Trimmer, J. S., and Rhodes, K. J. (2000) Nature 403, 553–556). We cloned a KChIP2 splice variant (KChIP2.2) from human ventricle. Comparison with KChIP2.1, coexpression of KChIP2.2 with human Kv4 channels in mammalian cells slowed the onset of Kv4 current inactivation (2–3-fold), accelerated the recovery from inactivation (5–7-fold), and shifted Kv4 steady-state inactivation curves by 8–29 mV to more positive potentials. The features of Kv4.2/KChIP2.2 currents closely resemble those of cardiac rapidly inactivating transient outward currents. KChIP2.2 stimulated the Kv4 current density in Chinese hamster ovary cells by ~55-fold. This correlated with a redistribution of immunoreactivity from perinuclear areas to the plasma membrane. Increased Kv4 cell-surface expression and current density were also obtained in the absence of KChIP2.2 when the highly conserved proximal Kv4 N terminus was deleted. The same domain is required for association of KChIP2.2 with Kv4 α-subunits. We propose that an efficient transport of Kv4 channels to the cell surface depends on KChIP binding to the Kv4 N-terminal domain. Our data suggest that the binding is necessary, but not sufficient, for the functional activity of KChIPs.

Voltage-gated potassium (Kv) channels related to the Shal (Kv4) gene family (2–5) mediate rapidly inactivating outward currents related to neuronal subthreshold A-type currents (4) as well as transient outward currents (Ito) in cardiac myocytes (6). Kv4 channel subunits have been localized immunocytochemically to somatodendritic areas in rat brain neurons (7). The high abundance of neuronal Kv4 channels in neuronal soma and dendrites suggests that Kv4 channels play an important role in postsynaptic signal integration. In fact, Kv4 channel activity may prevent action potential back-propagation into dendrites, thereby controlling potentiation of dendritic excitability (8) and acting as “dendritic shock-absorbers” (9). Conversely, inactivation of Kv4 channels below the action potential firing threshold leads to increased postsynaptic neuronal excitability (10, 11).

Recently, small Ca2+-binding proteins and channel-interacting proteins (KChIPs) were discovered (1). They are encoded in three different genes (KChIP1, KChIP2, and KChIP3) (1) and are members of the recoverin superfamily of Ca2+-binding proteins (12), being closely related to frequenin (13). KChIPs are associated with Kv4 channels as shown by immunocytochemical colocalization and co-immunoprecipitation studies (1). Since KChIPs are prominently expressed in brain, neuronal Kv4 channels most likely contain KChIPs as an integral subunit component.

Kv4 channels are also expressed in cardiac tissue, where they mediate Ito. This current contributes to the early phase of cardiac action potential repolarization (14). We considered the possibility that, like neuronal Kv4 channels, cardiac Kv4 channels might be associated with KChIPs. Accordingly, we searched human cardiac mRNA for KChIP mRNA. In this work, we report the cloning of a human cardiac Kv4 channel cDNA, KChIP2.2, which represents a splice variant of KChIP2.1. Like KChIP2.1 (1), the coexpression of KChIP2.2 with Kv4 channels in transiently transfected tissue culture cells alters their voltage gating and inactivation properties and leads to an up to 100-fold increase in Kv4 channel cell-surface expression.

The modulatory effects of KChIP2.2 are due to a direct interaction of KChIP2.2 with a distinct N-terminal domain of Kv4 α-subunits, which was identified by a combination of biochemical experiments and electrophysiological examinations of Kv4α mutants coexpressed with KChIP2.2. The KChIP-binding domain is highly conserved among Kv4.1, Kv4.2, and Kv4.3 subunit sequences (15). Deletion of the KChIP-binding domain in Kv4 channels led to an increase in Kv4 current density comparable to that seen after coexpression of Kv4 with KChIP2.2. Our results are consistent with the observation that the Kv4 N-terminal domain and its interaction with KChIP2.2 are critical determinants of Kv4 channel trafficking to the cellular plasma membrane.

EXPERIMENTAL PROCEDURES

Cloning of KChIP2.2 cDNA—A DNA fragment encoding an N-terminal splice variant of human Kv4 (GenBankTM/EBI accession number AF199598) was amplified by standard reverse transcription-PCR protocols from total RNA of human ventricle (see below) using primers CCGGGATCCACCATGCGGGGCCAGGGCCGCAAG (sense) and CTGGGACCTGGCTAGATCAAGTGTT (antisense).

The abbreviation used is: KChIPs, Kv channel-interacting proteins; PCR, polymerase chain reaction; HA, hemagglutinin; CHO, Chinese hamster ovary; pF, picofarad; HEK, human embryonic kidney; MO, mouse; CMV, cytomegalovirus promoter.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF347114.
and ATAGAAGTCCGCGCCGCTAGATGACATGGTCAAGAGC (anti-sense), which contained BamHI and NotI restriction sites, respectively, at their 5’-ends. In addition, the sense primer contained a Kozak site (16) preceding the start codon. A single PCR product was obtained. After digestion with BamHI and NotI, it was subcloned into the mammalian expression vector pcDNA3 (Invitrogen). Sequencing revealed that the subcloned PCR fragment differed from the published KChIP2 sequence and represents a splice variant, which we named KChIP2.2 (GenBank™/EBI accession number AF347114). A neuronal splice variant of KChIP2, KChIP2.1, which corresponds to the published KChIP2 sequence (1), was cloned from the total RNA of human cerebral cortex as described above.

Cloning of Modified Kv4 and KChIP Constructs—In this study, we used the human Kv4.1 and Kv4.2 α-subunits and the shorter splice variant of the human Kv4.3 α-subunit (15). In addition to their wild-type forms, mutant channel constructs with 39-amino acid (Kv4.3Δ2–39) and 40-amino acid (Kv4.1Δ2–40) N-terminal deletions were created for functional expression studies, similar to Kv4.2Δ2–40 (17). Further truncation (Kv4.2Δ2–52) (17) yielded no functional expression. Kv4.2 mutants with shorter N-terminal deletions (Kv4.2Δ2–32, Kv4.2Δ2–20, and Kv4.2Δ2–10) were constructed using standard PCR techniques. Amino-terminal primers carried a Kozak site followed by a translation initiation codon carboxyl-terminal primers carried a termination codon. For immunoprecipitation experiments, cDNAs encoding the amino terminus, the buffers contained either 1 mM CaCl2 or 2 mM EGTA. The C termini of Kv4.2 and Kv4.2Δ2–20 (amino acids 21–180), were constructed similarly.

For immunoprecipitation experiments, cDNAs encoding the amino terminus carboxyl-terminal primers carried a termination codon. A single PCR product was obtained. For immunoprecipitation experiments, cDNAs encoding the amino terminus carboxyl-terminal primers carried a termination codon. A single PCR product was obtained.

RESULTS

KChIP2.2, a Novel KChIP2 Splice Variant in Cardiac Tissue—We used total RNA from human ventricle as a template to amplify cardiac KChIP cDNA by reverse transcription-PCR with primers deduced from the published KChIP2.1 sequence (1). We obtained an alternative human cardiac KChIP2 cDNA sequence (KChIP2.2). Alignment of the KChIP2.1 cDNA sequences with the GenBank™/EBI htsg Data Bank identified a sequence with the GenBank™/EBI accession number AF347114. A neuronal splice variant, KChIP2.2, which corresponds to the published KChIP2 sequence (1), was cloned from the total RNA of human cerebral cortex as described above.

Electrophysiological Recordings and Data Analysis—During experiments, the cells were constantly superfused with an extracellular solution containing 135 mM NaCl, 5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 5 mM d-glucose, 10 mM HEPES, 2 mM ATP, and 2 mM glutathione (pH 7.2) (KOH). Currents were recorded with an EPC9 patch-clamp amplifier (HEKA, Lambrecht, Germany) and the program package PULSE + PULSEFIT (HEKA) was used for data acquisition and analysis. Series resistance compensation was maximal (usually between 80 and 90%). Whole-cell configurations with series resistance values above 6 were not used for recordings. Activation and inactivation kinetics of Kv4-mediated currents were fitted simultaneously with Hodgkin-Huxley-related formalism (PULSE-FIT) as described previously (18). With this fitting procedure, the Kv4 current inactivation time course was approximated by two time constants, \( \tau_1 \) and \( \tau_2 \). The voltage dependences of steady-state activation and inactivation were fitted with a Boltzmann function of the form

\[
\frac{G_{\text{max}}}{1 + \exp((V - V_{\text{1/2}})/k)}
\]

where \( V_{\text{1/2}} \) is the potential for half-maximal activation (\( V_{\text{1/2}} \)) or inactivation (\( V_{\text{1/2}} \)), and the steepness of the voltage dependence is defined by the slope factor \( k \). The kinetics of the recovery from inactivation were fitted with a single exponential function. Pool data were given as means ± S.E., and statistical analysis was done with GraphPAD InStat using unpaired t tests with Welch correction for unequal S.D. values when necessary.

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contig harboring the KChIP2 gene (accession number AC010789) and revealed that KChIP2.2 is a KChIP2 splice variant lacking the second exon in the KChIP2.1 open reading frame (data not shown). As a consequence, the KChIP2.2 open reading frame was shorter, and a stretch of 32 amino acid residues in the N terminus was absent (Fig. 1). The derived KChIP2.2 protein sequence was shorter, lacking KChIP2.1 amino acid residues 25–67. For functional characterization, KChIP2.2 cDNA was cloned into a mammalian expression vector as described under “Experimental Procedures.”

**Coexpression with KChIP2.2 Increases Kv4 Current Density in CHO Cells**—Currents mediated by Kv4.2 channels in transiently transfected CHO cells were activated with standard voltage-jump protocols. In agreement with previous data (2–5, 17), Kv4.2 channels mediated typical A-type potassium currents (Fig. 2, A and B). Current densities (pA/pF) were estimated from the peak amplitudes of Kv4.2 currents that were recorded from each cell. The average Kv4.2 current density was very low (38 ± 9 pA/pF, n = 6) (Fig. 2C). In the presence of KChIP2.2, however, the Kv4.2 current densities were dramatically increased (2045 ± 207 pA/pF, n = 10) (Fig. 2, B and C). This represents a 55-fold stimulation, in comparison with a 9-fold increase in current density previously reported for coexpression of rat Kv4.2 with KChIP2.1 in CHO cells (1).

**Effects of KChIP2.2 on Kv4.2 Gating Parameters**—The effects of KChIP2.2 on biophysical Kv4.2 channel properties were investigated in transiently transfected HEK 293 cells. Coexpression of KChIP2.2 with Kv4.2 channels mainly affected the inactivation characteristics of Kv4.2 currents (Fig. 3, A–D; and Table I). The activation time constants at +40 mV showed only a small difference (Kv4.2 alone: $\tau_{\text{act}} = 0.72 ± 0.06$, n = 7; Kv4.2 + KChIP2.2: $\tau_{\text{act}} = 1.08 ± 0.06$, n = 10). By contrast, the inactivation time course was markedly slowed (Fig. 3A). The value for $\tau_{i}$, the predominant component of Kv4.2 channel inactivation with a weighted amplitude of 91.1 ± 0.6% (n = 12), was increased ~3-fold (Kv4.2 alone: $\tau_{i} = 17.5 ± 1.4$, n = 22; Kv4.2 + KChIP2.2: $\tau_{i} = 53.5 ± 3.5$, n = 10). $\tau_{i}$, which represents a minor component in Kv4.2 channel inactivation, was not significantly affected by KChIP2.2 (Table I). Like Kv4.2P1.1 (1), KChIP2.2 accelerated at ~80 mV the recovery of Kv4.2 channels from inactivation (Fig. 3B). The recovery time constants were $\tau_{\text{rec}} = 285 ± 21$ ms (n = 4) for Kv4.2 alone and $\tau_{\text{rec}} = 46 ± 7$ ms (n = 9) for Kv4.2 + KChIP2.2. Furthermore, the presence of KChIP2.2 shifted to more depolarized potentials both the midpoint for Kv4.2 of voltage activation (Kv4.2 alone: $V_{\text{1/2,act}} = -1.2 ± 2.2$ mV, n = 8; Kv4.2 + KChIP2.2: $V_{\text{1/2,act}} = +7.7 ± 2.7$ mV, n = 4) (Fig. 3, C and D; and Table I) and the midpoint for the voltage dependence of steady-state inactivation (Kv4.2 alone: $V_{\text{1/2,inact}} = -57.4 ± 1.4$ mV, n = 6; Kv4.2 + KChIP2.2: $V_{\text{1/2,inact}} = -41.5 ± 2.1$ mV, n = 4) (Fig. 3, C and D; and Table I). The overlap between the voltage activation and the steady-state inactivation curves defines a “conductance window,” i.e. the narrow region of negative potentials in which Kv4.2 channels conduct in the steady state (Fig. 3D). In the presence of KChIP2.2, the Kv4.2 conductance window was enlarged, and the point of maximal Kv4.2 channel availability...
TABLE I
Gating parameters for wild-type Kv4.2 and N-terminal deletion mutant Kv4.2Δ2–40 alone and coexpressed with KChIP2.2 in HEK 293 cells

| Condition          | WT-Kv4.2 | WT-Kv4.2 + KChIP2.2 | Kv4.2Δ2–40 | Kv4.2Δ2–40 + KChIP2.2 |
|--------------------|----------|---------------------|------------|----------------------|
| \( \tau_{\mathrm{act}} \) (ma) | 0.72 ± 0.06 (n = 7) | 1.08 ± 0.06 (n = 10) | 0.77 ± 0.09 (n = 6) | 0.94 ± 0.06 (n = 18) |
| \( \tau_{\mathrm{inact}} \) (ma) | 17.5 ± 1.4 (n = 22) | 55.3 ± 3.5 (n = 10) | 62.3 ± 3.4 (n = 18) | 75.8 ± 4.1 (n = 18) |
| \( \tau_{\mathrm{rec}} \) (ma) | 232 ± 17 (n = 22) | 238 ± 24 (n = 10) | 211 ± 11 (n = 18) | 232 ± 13 (n = 18) |
| \( \% \tau_{\mathrm{inact}} \) | 91.1 ± 0.6 (n = 22) | 89.3 ± 0.6 (n = 10) | 67.6 ± 2.8 (n = 18) | 67.4 ± 2.9 (n = 18) |

\( ^a \) Significantly different from control with \( p < 0.0001 \).
\( ^b \) Significantly different from control with \( p < 0.04 \).

FIG. 5. Co-immunoprecipitation of Myc-tagged KChIP and Kv4.2 amino termini. \(^{35}\)S-Labeled Myc-tagged KChIP2.2 and Kv4.2 N termini (Kv4.2-NT, Kv4.2-NTΔ2–10, and Kv4.2-NTΔ2–20) were obtained by \textit{in vitro} co-translation. Co-translated polypeptides were co-immunoprecipitated with anti-Myc antibodies in the presence of 1 \textmu M CaCl\(_2\) (+) or 2 \textmu M EGTA (−). Polypeptides were analyzed on 15% SDS-polyacrylamide gel and visualized by autoradiography. Pre-IP, in \textit{vitro} co-translated mixture of \(^{35}\)S-labeled Myc-tagged KChIP2.2 and Kv4.2 N termini before immunoprecipitation; IP, immunoprecipitated \(^{35}\)S-labeled polypeptides. The arrows indicated the positions of \(^{35}\)S-labeled KChIP2.2-Myc and Kv4.2 N terminus. Molecular weight markers are on the left.

Fig. 4. N-terminal truncation interferes with KChIP2.2 effects on Kv4.2. A, current densities at +40 mV in CHO cells measured for wild-type (wt) Kv4.2 and different N-terminal deletion mutants (Kv4.2Δ2–10, Kv4.2Δ2–20, Kv4.2Δ2–32, and Kv4.2Δ2–40) in the absence (black bars) and presence (white bars) of KChIP2.2. Numbers of cells are indicated in parentheses. Error bars represent S.E. Note that in the absence of KChIP2.2, N-terminal truncation increased current densities. B, data shown in A expressed as the factor by which the current density was increased either by N-terminal truncation (black bars; mean current densities obtained with N-terminal deletion mutants divided by the mean current density obtained with the wild type) or in the presence of KChIP2.2 (white bars; mean current densities obtained in the presence of KChIP2.2 divided by the mean current densities obtained without KChIP2.2 for each Kv4.2 channel construct). The dotted line represents unity (no change in current density, as observed for Kv4.2Δ2–20, Kv4.2Δ2–32, and Kv4.2Δ2–40 in the presence of KChIP2.2). C, representative current trace recorded from a HEK 293 cell cotransfected with Kv4.2Δ2–40 and KChIP2.2. The stimulation protocol was as described for Fig. 3A. The idealized current trace (dotted line) is based on the mean fitting parameters obtained for Kv4.2Δ2–40 alone (Table I). The normalized traces are almost identical. D, recovery of Kv4.2Δ2–40 channels from inactivation. ○, Kv4.2Δ2–40 alone; □, Kv4.2Δ2–40 coexpressed with KChIP2.2. Single exponential fitting (dotted line for Kv4.2Δ2–40 alone) of the data yielded similar curves.

was shifted by ~15 mV to more positive membrane potentials (Fig. 3D). In summary, the coexpression of KChIP2.2 with Kv4.2 increased Kv4.2 current density, slowed the inactivation time course of Kv4.2 currents, accelerated the recovery of Kv4.2 channels from inactivation, and increased the voltage range where Kv4.2 channels can be activated under steady-state conditions.

Distinct Kv4.2 N-terminal Domain Critical for KChIP2.2 Function—We investigated the possibility that the observed effects of KChIP2.2 on Kv4.2 currents might depend on distinct Kv4.2 domain(s). In a first approach, the Kv4.2 N-terminal deletion mutant Kv4.2Δ2–40 (17) was used. Compared with the wild type, transient transfection of CHO cells with the truncated Kv4.2 construct Kv4.2Δ2–40 yielded significantly increased current densities (Kv4.2: 38 ± 9 pA/pF, \( n = 6 \); Kv4.2Δ2–40: 1129 ± 60 pA/pF, \( n = 10 \)) (Fig. 4A). Coexpression of Kv4.2Δ2–40 with KChIP2.2 did not produce an additional increase in current density (Kv4.2Δ2–40 + KChIP2.2: 1093 ± 118 pA/pF, \( n = 9 \)) (Fig. 4A). Smaller truncations of the Kv4.2 N terminus, e.g., Kv4.2Δ2–32 and Kv4.2Δ2–20, had effects similar to those observed with Kv4.2Δ2–40 (Fig. 4, A and B), i.e., the current densities in transiently transfected CHO cells had increased, albeit to a lesser degree. At the same time, the stimulatory effect of KChIP2.2 was attenuated. However, expression of the Kv4.2Δ2–10 construct yielded current densities similar to those of the wild-type (85 ± 17 pA/pF, \( n = 9 \)), and the presence of KChIP2.2 increased Kv4.2Δ2–10 current density ~6-fold (497 ± 107 pA/pF, \( n = 10 \)) (Fig. 4, A and B).
was slowed in comparison with wild-type Kv4.2, resulting in an ~3-fold increase in the inactivation time constant $\tau_i$ (Kv4.2: $\tau_i = 17.5 \pm 1.4 \text{ ms}$, $n = 22$; Kv4.2Δ2–40: $\tau_i = 62.3 \pm 3.4 \text{ ms}$, $n = 18$). The inactivation time constant $\tau_2$ remained unchanged (Table I), but the weighted amplitude of $\tau_2$ had increased to $32.4 \pm 2.8\%$ ($n = 18$) for Kv4.2Δ2–40 in comparison with $8.9 \pm 3.5\%$ ($n = 12$) for Kv4.2. Recovery kinetics of Kv4.2Δ2–40 channels from inactivation at $-80 \text{ mV}$ ($\tau_{rec} = 260 \pm 80 \text{ ms}$, $n = 3$) were indistinguishable from those of Kv4.2 (Fig. 4D and Table I). Also, the midpoints of voltage activation ($V_{1/2,act}$) and of steady-state inactivation ($V_{1/2,inact}$) did not differ significantly between Kv4.2 and Kv4.2Δ2–40 channels (Table I). Coexpression with KChIP2.2 affected neither kinetic nor steady-state parameters of Kv4.2Δ2–40 currents (Table I). These results suggest that KChIP2.2 does not interact with Kv4.2Δ2–40 channels.

**Physical Association of KChIP2.2 with Kv4.2 N Terminus**—To investigate the physical association of KChIP2.2 with the Kv4.2 N terminus, we carried out co-immunoprecipitation experiments. The Kv4.2 N terminus (amino acid residues 1–180; designated as Kv4.2-NT) and C-terminally Myc-tagged KChIP2.2 (KChIP2.2-Myc) were cotranslated *in vitro* and $^{35}$S-labeled. As shown in Fig. 5, anti-Myc antibodies co-immunoprecipitated Kv4.2-NT with KChIP2.2-Myc. Similar co-immunoprecipitation results were obtained with cotranslated KChIP2.2-Myc and Kv4.2-NTΔ2–10, a Kv4.2-NT construct truncated by the first 10 amino acid residues. Incubation of the cotranslated KChIP2.2/Kv4.2-NT polypeptide mixture with 1 mM CaCl$_2$ or 2 mM EGTA did not influence the co-immunoprecipitation results, in agreement with a previous report that the association of KChIP1 with Kv4.2 is calcium-insensitive (1). Anti-Myc antibodies did not co-immunoprecipitate to a significant extent KChIP2.2-Myc with Kv4.2-NTΔ2–20, which corresponds to a Kv4.2-NT construct truncated by 20 amino acid residues (Fig. 5).

To further support our notion that the Kv4.2 N terminus is critical for association of KChIP2.2 with full-length Kv4.2 α-subunits, Kv4.2 and KChIP2.2-Myc were transiently expressed alone or together in CHO cells. We tagged Kv4.2 with a HA epitope to monitor the expression of Kv4.2 by immunofluorescent staining with anti-HA antibodies. KChIP2.2-Myc was stained with an anti-Myc antibody. When expressed alone, Kv4.2 was concentrated within the perinuclear endoplasmic reticulum and Golgi compartments, with some immunoreactivity apparent in the outer margins of the cell (Fig. 6A) as previously reported (1). Anti-Myc antibodies did not co-immunoprecipitate to a significant extent KChIP2.2-Myc with Kv4.2-NTΔ2–20, which corresponds to a Kv4.2-NT construct truncated by 20 amino acid residues (Fig. 5).

**Conserved Kv4 N-terminal Domain Critical for KChIP2.2 Function**—The data shown in Fig. 7 demonstrate that Kv4.1 and Kv4.3 current densities were also significantly increased in transiently transfected CHO cells upon coexpression with KChIP2.2 (Kv4.1 alone: 1 ± 1 nA/pF, $n = 3$; Kv4.1 + KChIP2.2: 1352 ± 443 nA/pF, $n = 5$; Kv4.3 alone: 159 ± 41 nA/pF, $n = 4$; Kv4.3 + KChIP2.2: 1637 ± 411 nA/pF, $n = 10$). Since the sequence of the first 23 amino acid residues of Kv4α N termini are highly conserved (15), we expected that the N termini of Kv4.1 and Kv4.3 would be of similar importance for binding

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**Fig. 6. Subcellular localization of Kv4.2 and Kv4.2Δ2–40 in CHO cells in the absence or presence of KChIP2.2.** A–C, transient expression of HA-tagged Kv4.2, HA-tagged Kv4.2Δ2–40, and Myc-tagged KChIP2.2 in CHO cells, respectively; D–F, coexpression of Kv4.2 with KChIP2.2; G–I, coexpression of Kv4.2Δ2–40 with KChIP2.2. Each micrograph is labeled at the top. Scale bars = 20 μm. aHA, detection with anti-HA antibody; anymc, detection with anti-Myc antibody; overlay, merged image.

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**Fig. 7. Effect of KChIP2.2 on Kv4.1 and Kv4.3 current densities.** Shown are current densities obtained at +40 mV in CHO cells expressing either Kv4.1 (A) or Kv4.3 (B) in the absence (black bars) and presence (white bars) of KChIP2.2. Both the wild type (wt) and N-terminal deletion mutants (Kv4.1Δ2–40 and Kv4.3Δ2–39) were tested. Wild-type Kv4.1 channels showed poor expression in three cells tested (asterisk) in the absence of KChIP2.2. Only coexpression with KChIP2.2 resulted in measurable currents. Numbers of cells are indicated in parentheses. Error bars represent S.E.
KChIP2.2. Indeed, N-terminal truncation of both Kv4.1 (Kv4.1Δ2–40) and Kv4.3 (Kv4.3Δ2–39) led to an increase in current density, which did not further increase in the presence of KChIP2.2 (Fig. 7, A and B). Kv4.1Δ2–40 and Kv4.3Δ2–39 current densities in transiently transfected CHO cells were comparable to those observed for the wild type in the presence of KChIP2.2.

Next, we determined the kinetics of Kv4.1 and Kv4.3 currents ± KChIP2.2. The results are summarized in Table II. As for Kv4.2 currents, KChIP2.2 did not markedly affect the activation time courses of Kv4.1 and Kv4.3 currents at +40 mV, but slowed the inactivation time course. The respective increase in the inactivation time constant τi was comparable to that observed upon coexpression of Kv4.2 with KChIP2.2 (Table I). In addition, the slower inactivation time constant τ2 was increased, as well as the weighted amplitudes of τ2. Furthermore, KChIP2.2 accelerated the recovery of Kv4.1 and Kv4.3 channels from inactivation and shifted the midpoints of voltage activation (V1/2,act) and of steady-state inactivation (V1/2,inact) to more depolarized membrane potentials (Table II). In summary, the effects of KChIP2.2 on Kv4.1 and Kv4.3 channel expression and gating parameters were very similar, but not identical to those of KChIP2.2 on Kv4.2.

**DISCUSSION**

We have identified a KChIP2 splice variant in mRNA isolated from human ventricle. In comparison with the KChIP2.1 protein sequence (Ref. 1 and Fig. 1), KChIP2.2 lacks KChIP2.1 amino acids 25–56 at the N terminus. The respective 96 base pairs of KChIP2.1 cDNA correspond to exon 2 of the KChIP2 open reading frame, according to a comparison of KChIP2.1 cDNA with human genomic sequences in the NCBI/GenBank®/EBI Data Bank (data not shown). Orthologous KChIP2.2 cDNA sequences in mouse (accession number AF044571) and rat (accession number AF269285) were detected by a BLAST search of the NCBI/GenBank®/EBI Data Bank. Reverse transcription-PCR results indicated that predominantly KChIP2.2 is expressed in cardiac tissue. Subsequent reverse transcription-PCR experiments with total RNA from human brain revealed that the mRNAs of both KChIP2 splice variants, KChIP2.1 and KChIP2.2, are expressed in comparable amounts in brain (data not shown).

**KChIP2.2 Stimulates Availability of Active Kv4 Channels in Plasma Membrane**—The coexpression of KChIP2.2 together with human Kv4.1, Kv4.2, or Kv4.3 in transiently transfected mammalian cells led to a significant increase in Kv4 current densities, slowed onset of inactivation, and accelerated recovery from inactivation. The results are in general agreement with those published for the effects of KChIP2.1 on rat Kv4.2 currents (1), but the effects mediated by KChIP2.2 and KChIP2.1, respectively, differ in detail. The stimulation of rat Kv4.2 current density by coexpression with KChIP2.1 was −9-fold (1). In experiments done with KChIP2.1, we observed a similar ~14-fold increase in human Kv4.2 current density (536 ± 89 pA/pF, n = 6), in comparison with an ~55-fold increase in Kv4.2 current density with KChIP2.2. The results suggest that the variable N terminus of KChIP2 influences the magnitude of the KChIP effect on current density. Furthermore, the value of the predominant inactivation time constant τi in the presence of KChIP2.1 was 43.6 ± 2.1 ms (n = 21) at +40 mV, significantly different both from Kv4.2 alone (p < 0.0001) and from Kv4.2 + KChIP2.2 (p = 0.0183) (see Table I). The mean time constant for the recovery from inactivation (τrec) at −80 mV was 52.3 ± 5.2 ms (n = 7) for Kv4.2 + KChIP2.1, faster than that for Kv4.2 alone (p < 0.0001), but not significantly different from that for Kv4.2 + KChIP2.2 (p = 0.5046) (see Table I). We also found that the voltage for half-maximal steady-state inactivation of Kv4.2 currents was shifted to more positive membrane potentials in the presence of KChIP2.1 (V1/2,inact = −50.3 ± 1.2 mV, n = 6; p = 0.0032), however, by a different degree as compared with KChIP2.2 (p < 0.0001) (see Table I). Thus, KChIP2.1 and KChIP2.2 distinctly altered Kv4 current properties. Nevertheless, the KChIP effects have in common an increased availability of active Kv4 channels by a rise in Kv4 channel density in the plasma membrane, slowed inactivation, an acceleration of the recovery from inactivation, and/or alteration of the operational range due to the change in the voltage dependence of steady-state inactivation.

**Kv4.2/KChIP2.2 Current Properties Resemble Cardiac Ito**—The results of Northern blot (6, 19) and immunohistochemical (20) studies suggest that Kv4.2 and Kv4.3 subunits are expressed in cardiac tissue, in particular in ventricle (5, 15). Since KChIP2.2, which we isolated from cardiac tissue, physically

| Kv4.1 | Kv4.1 + KChIP2.2 | Kv4.3 | Kv4.3 + KChIP2.2 |
|-------|-----------------|-------|-----------------|
| τact (40 ms) (μs) | 1.38 ± 0.12 (n = 11) | 0.86 ± 0.03 (n = 13) | 1.147 ± 0.112 (n = 11) |
| Δτact (40 ms) (μs) | 58.5 ± 4.1 (n = 11) | 23.2 ± 2.5 (n = 13) | 61.9 ± 5.0 (n = 11) |
| Δτinact (40 ms) (μs) | 45.0 ± 9.8 (n = 5) | 269 ± 20 (n = 5) | 60 ± 5 (n = 5) |
| V1/2,act (mV) | −35.8 ± 2.0 (n = 5) | −54.6 ± 2.2 (n = 4) | −38.5 ± 1.6 (n = 6) |
| kact (mV) | 4.5 ± 0.2 (n = 5) | 5.1 ± 0.2 (n = 4) | 4.1 ± 0.2 (n = 6) |
| V1/2,inact (mV) | −6.6 ± 2.9 (n = 4) | −4.1 ± 2.1 (n = 7) | +10.2 ± 2.1 (n = 7) |
| krec (mV) | 15.5 ± 1.2 (n = 4) | 16.6 ± 1.6 (n = 5) | 16.7 ± 0.7 (n = 7) | 15.9 ± 0.6 (n = 7) |

*Significantly different from control with p < 0.0001.
+Significantly different from control with p < 0.01.
associates with Kv4 channels, Kv4/KChIP2.2 heteromultimer expression may reconstitute native A-type Kv channels that mediate the rapidly inactivating transient outward current ($I_{to}$), a key component in the early repolarizing phase of cardiac action potentials (14). Indeed, the features that we determined for Kv4.2/KChIP2.2 currents resemble very closely those that have been determined for $I_{to}$ in neonatal rat ventricular myocytes ($I_{to}$; $\tau_3 = 49 \pm 13$ ms, $\tau_{rec} = 86 \pm 38$ ms, $V_{i50,\text{inact}} = -37 \pm 3$ mV (21); Kv4.2/KChIP2.2: $\tau_3 = 53.3 \pm 1.4$ (n = 22), $\tau_{rec} = 46 \pm 7$ (n = 22), $V_{i50,\text{inact}} = -41.5 \pm 2.1$ mV).

Proximal N-terminal Domain Is Critical for Kv4 Channel Surface Expression—The N termini of Shaker-related A-type channels like Kv1.4 contain a characteristic inactivating domain that is responsible for the rapid inactivation behavior of these Kv channels (22). Kv4 channels also inactivate rapidly, but the mechanism of inactivation is quite different and does not depend on the presence of an N-terminal Shaker-like inactivating domain (23, 24). Yet, the Kv4 N terminus also contains a highly conserved 20-amino acid-long domain (Fig. 8) (15). The domain is characteristic for Kv4 N terminus and is not found in other Kv subunit sequences. Our results indicate that the conserved Kv4 N-terminal domain may have important functions correlated with cell-surface expression of Kv4 channels and with binding KChIPs. The observed effects of KChIPs on Kv4 channel surface expression may be due to facilitated trafficking to the plasma membrane, delayed Kv channel turnover, altered interactions with the cytoskeleton, and/or alteration in intrinsic functional properties of the ion channel complex. We favor the notion that the Kv4 N terminus participates in regulation of Kv4 channel trafficking to the cell surface for the following reasons. In agreement with previous data (1), the Kv4.2 immunostaining pattern in the transiently transfected CHO cells showed an intense perinuclear Kv4.2 immunoreactivity in the absence of KChIP2.2. This is consistent with a retention of Kv4.2 protein in the endoplasmic reticulum or Golgi compartments. Deletion of the Kv4.2 N terminus or coexpression of Kv4.2 with KChIP2.2 produced a subcellular redistribution of Kv4 immunoreactivity to the cell membrane. Furthermore, our results showed that deletion of the Kv4.2 N terminus decreased the functional sensitivity of Kv4.2 channels to coexpressed KChIP2.2, most likely due to a loss of the KChIP-binding site on the Kv4 N terminus. Deletion of the conserved N terminus ablated KChIP2.2 binding to the Kv4 N terminus as well as KChIP2.2-mediated increases in current densities. At the same time, the deletions of the Kv4 N terminus yielded a KChIP-independent increase in Kv4 channel density at the cell surface. Taken together, our observations strengthen the possibility that the Kv4 N terminus may contain an endoplasmic reticulum retention signal. Then, deletion of the Kv4 N terminus might attenuate the endoplasmic reticulum retention signal and accordingly stimulate a redistribution of Kv4 channels to the cell surface. Similarly, binding of KChIPs to the Kv4 N terminus may facilitate Kv4 channel trafficking to the cell surface by masking the endoplasmic reticulum retention signal.

A comparison of the expression data for Kv4.2 deletion mutants with Kv4.2/KChIP2.2 coexpression data shows some significant qualitative and quantitative differences with respect to current density increase. The deletions of the Kv4.2 N terminus gave rise to an up to ~30-fold KChIP-independent increase in Kv4.2 current density as compared with ~14- and ~55-fold increases in Kv4.2 current density with coexpressed KChIP2.1 and KChIP2.2, respectively. Thus, binding of KChIPs to the Kv4.2 N terminus, as well as Kv4.2 N-terminal deletion, may cause different increases in current density. The data indicate, as discussed above, that the functional activity of KChIPs may be modulated by their variable amino termini. Furthermore, KChIP binding to the Kv4.2 N terminus is not Ca$^{2+}$-dependent in vitro, but the functional activity of KChIPs is most likely Ca$^{2+}$-sensitive because it requires intact EF-hands (1). Obviously, binding of KChIP to the Kv4 N terminus is necessary, but not sufficient, for functional KChIP activity. Future studies have to show whether the various mechanisms by which KChIPs regulate Kv4 channel activity involve distinct structures of the KChIP molecule, e.g. amino terminus, conserved core region, and Ca$^{2+}$-binding sites.

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