Analysis of the Cyclic Nucleotide Binding Domain of the HERG Potassium Channel and Interactions with KCNE2*

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Mutations in the cyclic nucleotide binding domain (CNBD) of the human ether-a-go-go-related gene (HERG) K+ channel are associated with LQT2, a form of hereditary Long QT syndrome (LQTS). Elevation of cAMP can modulate HERG K+ channels both by direct binding and indirect regulation through protein kinase A. To assess the physiological significance of cAMP binding to HERG, we introduced mutations to disrupt the cyclic nucleotide binding domain. Eight mutants including two naturally occurring LQT2 mutants V822M and R823W were constructed. Relative cAMP binding capacity was reduced or absent in CNBD mutants. Mutant homotetramers carry little or no K+ current despite normal protein abundance and surface expression. Co-expression of mutant and wild-type HERG resulted in currents with altered voltage dependence but without dominant current suppression. The data from co-expression of V822M and wild-type HERG best fit a model where one normal subunit within a tetramer allows nearly normal current expression. The presence of KCNE2, an accessory protein that associates with HERG, however, conferred a partially dominant current suppression by CNBD mutants. Thus KCNE2 plays a pivotal role in determining the phenotypic severity of some forms of LQT2, which suggests that the CNBD of HERG may be involved in its interaction with KCNE2.

Long QT syndrome is an inherited cardiac disorder that causes sudden death from tachyarrhythmias. Five loci have been mapped to 11p15.5 (LQT1), 7q35-36 (LQT2), 3p21-24 (LQT3), 4q25-27 (LQT4), and 21q22-23 (LQT5 and LQT6) (1). Several other kindreds have not been fully mapped. The gene responsible for LQT2 is the human ether-a-go-go-related gene (HERG), which encodes the pore-forming subunit of the rapidly activating delayed rectifier potassium channel (Ikr) in cardiac myocytes (2, 3). MirP1, the gene product of KCNE2 (LQT6) and minK, the gene product of KCNE1 (LQT5), are small membrane proteins that are capable of assembling with HERG and regulating its function (4, 5).

The HERG protein contains a putative cyclic nucleotide binding domain (CNBD) in its cytoplasmic carboxyl terminus (2). A splice-acceptor mutation resulting in a C-terminal truncation lacking the entire CNBD was one of the HERG mutants that was originally identified as a cause of LQT2 (3). More recently, new LQT2 mutations have been discovered in the putative CNBD (1, 6, 7).

Although the cardiac Ikr was originally thought to be insensitive to cAMP-dependent regulation (3, 8), recent studies provided evidence that HERG channels can be modulated via cAMP-dependent protein kinase (PKA) phosphorylation pathway (9–11). We reported that cAMP could regulate HERG by direct interaction or through PKA-mediated phosphorylation of the channel (12). In this study, we further investigate the direct effect of cAMP on HERG channel properties through mutagenesis designed to disrupt the ability of HERG to bind cAMP.

Previous studies of the CNBD in cyclic nucleotide-dependent protein kinase and bacterial cAMP-regulated catabolic gene activator protein (CAP) identified six invariant key amino acid residues for cyclic nucleotide binding (13). Three of these are glycine residues that are essential for maintenance of the β-barrel structure that is required to form a pocket for cyclic nucleotide binding. A glutamic acid residue forms a hydrogen bond with the ribose 2′-OH of cAMP and an arginine residue interacts with the phosphate of cAMP to form a salt bridge (14). Mutagenesis of any of these six invariant amino acid residues in CAP or in type I cAMP-dependent kinase imparis or eliminates cAMP binding (15–20). Homologous amino acid residues are also present in the CNBD of HERG. Here we describe the functional consequences of mutations at these homologous residues (G806D, E807(K/Q), and R823(W/Q)) designed to alter the ability of the channel to bind cAMP. Because HERG assembles in a tetramer to form functional channels, we also investigated the effects of coexpression of CNBD mutants with wild-type HERG. We found that CNBD mutants lose specific binding affinity for cAMP. They do not express currents as homotetrameric channels. Moreover, the loss of function is not because of defective protein biosynthesis or trafficking. Mutant HERG proteins do not have a dominant-negative effect on wild-type current but do alter voltage-dependent gating. Coexpression with the accessory subunit KCNE2, converts the CNBD mutants into partially dominant-negative suppressors.

MATERIALS AND METHODS

Mutagenesis of HERG cDNA—Epitope tagging of HERG with c-Myc has been described (4). Site-directed missense mutagenesis was performed by an overlap extension PCR strategy (21). The mutated XhoI-FseI fragments were subcloned into a HERG-myc-pCI-neo plasmid. An intrame deletion mutation ΔNBD was constructed using a one-step PCR. The forward primer begins at 2662 of HERG cDNA (5′-TACTGTTG)

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1 The abbreviations used are: HERG, human ether-a-go-go-related gene; LQTS, Long QT Syndrome; CNBD, cyclic nucleotide binding domain; PKA, cAMP-dependent protein kinase; GFP, green fluorescent protein; Ikr, rapidly activating delayed rectifier potassium current; CHO, Chinese hamster ovary; GPT-cAMP, 5-chlorophenylthio-adenosine 3′,5′-cyclic monophosphate; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; HEK, human embryonic kidney; WT, wild-type.
Acid (pH 7.2). Osmolarity was adjusted to maintain the 120 mM KCl, 4 mM Mg-ATP, 2 mM MgSO\(_4\), 5 mM EGTA, 0.5 mM CaCl\(_2\), 120 mM potassium phosphate, 10 mM MgCl\(_2\), 1 mg/ml bovine serum albumin, 0.5 mg/ml Histone I, 2 mM EDTA, 1 mg/ml bovine serum albumin, 0.5 mg/ml Histone I, and 2 mM MgCl\(_2\). Cells were electroporated at 250 V, 72 ohms, and 1800 microfarads in cytomix media. Cells were studied after 36–72 h after transfection. A plasmid containing the cDNA for GFP was combined with HERG plasmids (in a ratio of 1:5 GFP/HERG) to allow identification of transfected cells as previously described (4). In co-transfection experiments current amplitudes were normalized each day to a group of cells transfected with HERG alone to control for day-to-day variations in transfection efficiency.

Electrophysiology—Whole-cell patch clamp (23) membrane currents were recorded as previously described (12). Extracellular solution was NaCl 150 mM, 1.8 mM CaCl\(_2\), 4 mM KCl, 1 mM MgCl\(_2\), 5 mM glucose, and 10 mM Hepes, pH 7.4. Osmolarity was adjusted to maintain the pipette solution at 20–40 mOsm less than the extracellular solution. Currents were evoked by 2–4 s depolarizing steps to various levels from a holding potential of −70 mV followed by a repolarizing step to −40 mV and then to −120 mV briefly to measure outward and inward tail currents. Current densities were calculated by dividing maximal tail current peaks by cell capacitance measurements. Voltage activation data were plotted as peak tail current amplitudes against the test potential values and were fitted to a Boltzmann function, \( I = \frac{1}{1 + \exp(V_m - V_0)/k} \), where \( I \) is the measured tail current, \( V \) is the applied membrane voltage, \( V_0 \) is the voltage at half-maximal activation, and \( k \) is the slope factor.

Immunoblot Analysis—CHO or HEK293 cells were transiently transfected using LipofectAMINE 2000 according to the manufacturer’s instructions (Life Technologies, Inc.). Cells were harvested 24 h after transfection for biochemical analyses. Membrane preparations were enriched for plasma membrane proteins and Western blots were carried out as previously described (4, 24). To control for transfection efficiency between groups of cells, cDNA for Myc-tagged connexin43 was included. Proteins were separated by SDS-PAGE on a 7% gel. Anti-Myc 9E10 monoclonal antibody ascites fluid was used for Western blots to detect Myc-tagged HERG proteins.

Antibodies—Anti-HERG polyclonal antisera was generated against a GST fusion protein constructed from a PCR fragment encoding amino acids 979–1159 ligated into pGEX-KG. The GST-HERG fusion protein was expressed in Escherichia coli strain BL21 and was partially purified on GSH-agarose. The protein was then identified by Coomasie Blue staining after SDS-PAGE, and the appropriate gel band was excised, electroeluted, and injected into New Zealand White rabbits. The resulting antiserum was assayed for ability to recognize heterologously expressed HERG-Myc protein on immunoblot and immunoprecipitation and was verified by comparison to anti-Myc monoclonal antibody staining. Anti-Myc monoclonal antibody was used as ascites fluid diluted into immunoblot blocking solution (Tris-buffered saline, 10% nonfat dry milk, and 0.1% Tween 20).

Microscopy—CHO cells were transiently transfected with LipofectAMINE (as above) with GFP/HERG plasmids. After 24–48 h, the transfected cells were resuspended and replated on glass coverslip chambers (Nunc) and allowed to attach for at least 24 h prior to imaging. GFP fluorescent images were collected using an Olympus IX70 microscope with 12 bit cooled Photometrics SenSys CCD camera. Image analysis was performed using NIH Image, ImagePro, and Photoshop 7.0 software.

cAMP Binding Assay—For \(^3\)H(cAMP binding, HERG-Myc was immunoprecipitated from HEK293 cell lysates with anti-Myc polyclonal antibody A-14 (Santa Cruz Biotechnology, Inc.) and Ultralink immobilized Protein A (Pierce). Precipitated proteins were washed with ice-cold NDEI (150 mM NaCl, 0.4% deoxycholic acid, 5 mM EDTA, 25 mM Tris, 1% Nonidet P-40) and suspended in cAMP binding buffer (50 mM Tris, 120 mM potassium phosphate, 10 mM MgCl\(_2\), 1 mg/ml bovine serum albumin, 0.5 mg/ml Histone I, 2 mM EDTA, 1 mg/ml isobutylmethylxanthine (IBMX), pH 7.0). Determination of cyclic nucleotide binding was performed as previously described (12).

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Targeted Mutations of HERG CNBD Result in Functionally Defective Channels—To investigate the importance of the HERG cyclic nucleotide binding domain, we constructed eight CNBD mutations targeted to disrupt cAMP binding (Fig. 1a). The selected mutant amino acid residues are homologous to invariant residues in other cyclic nucleotide-binding proteins that have been identified as essential for normal conformation and cAMP binding ability. Among these mutations, V822M and R823W are naturally occurring LQT2 mutations (1, 6). ΔNBD is an inframe deletion mutant HERG lacking 11 amino acids in the cyclic nucleotide binding site (Δ815–825). We measured the whole cell K\(^+\) current from CHO cells transiently transfected with each mutant transcript. Typical whole cell current tracings are shown in Fig. 1b. Except for mutant R823Q, none of the mutations could produce voltage-gated K\(^+\) current when expressed alone. We also did not detect any K\(^+\) current when mutant expressing cells were exposed to 1 mM CPT-cAMP. Our results confirmed previous reports that LQT2 homotetramers involving the cyclic nucleotide binding domain (V822M and S818L) do not form functional channels (25, 26).

One of the CNBD mutants, however, R823Q, did produce HERG current. Comparison of current-voltage and voltage-dependent activation showed that the current amplitude was greater for wild-type HERG than R823Q homotetramers at all voltages (Fig. 2a). The maximal tail current density measuring at −40 mV after a 2 s depolarizing test for wild-type HERG is 54.5 ± 5.8 pA/pF (n = 8); whereas R823Q is 11.3 ± 1.37 pA/pF (n = 3; p < 0.001). When the data were independently normalized to unitary (Fig. 2a, inset), the voltage to achieve half-activation (\(V_{1/2}\)) for R823Q was −12.33 ± 0.23 mV; \(V_{1/2}\) WT = −12.09 ± 0.31 mV. There was no apparent change in the slope factor (\(k_{WT}\) = 10.13 ± 0.21 mV; \(k_{R823Q}\) = 9.21 ± 0.29 mV).

We have previously shown that cAMP decreased whole cell HERG current amplitude with minimal alteration in voltage-dependent activation (12). This current inhibition was primar-
HERG Cyclic Nucleotide Binding Domain and KCNE2 Interactions

**Fig. 2. Electrophysiological properties of mutant R823Q.** (a) Comparison of current density of wild-type (WT) HERG (○), and mutant R823Q (●). Both current-voltage (left graph) and voltage-activation curves (right graph) show that current density is greater for wild-type HERG than mutant R823Q at all voltages. *Inset*, data normalized to unity showing the hyperpolarizing shift in voltage-dependent activation. (b) Effects of cAMP on R823Q K⁺ current. Current-voltage (left graph) and voltage-activation curves (right graph) show that there was a 15% current reduction and a left shift in voltage dependence of activation after cAMP treatment. *Inset*, data normalized to the maximum current in the same curve.

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Analysis of CNBD Mutant HERG Protein Expression—Several LQT2 mutations are known to cause reduced Iₐ,K by defective transport of mutant proteins to the plasma membrane or through enhanced protein degradation (24, 25, 27, 28). When misfolded mutant HERG subunits assemble with wild-type protein, a dominant effect can be exerted where both proteins are subjected to early and rapid degradation (24). To assess whether the dysfunction of CNBD mutations was also caused by abnormal protein biosynthesis or trafficking, we examined the abundance and the membrane expression of c-Myc-tagged HERG and CNBD mutants. Anti-Myc immunoblot analysis showed that the CNBD mutants were expressed in abundance less than but comparable with wild-type HERG (24). As shown in Fig. 3c, both wild-type HERG and the CNBD mutants were expressed as two bands on Western blot. In all groups, both bands migrated slightly slower when grown at 30 °C. No enhancement of mutant protein abundance occurred in cells that were grown at the lower temperature. Moreover, we were not able to detect any HERG current in CHO cells transfected with the V822M or ΔNBD mutants after incubation at 30 °C for 36–72 h. The R823W mutant, however, occasionally produced a very small amount of current when the incubation temperature was decreased to 27–30 °C for 24–48 h. Among 12 examined cells, 6 exhibited small HERG currents (Fig. 3d). The average tail current density for wild-type and R823W at 30 °C was 117.6 ± 14.5 pA/pF and 8.25 ± 2.7 pA/pF, respectively (p < 0.01). Despite comparable protein abundance, low temperatures resulted in a current that was expressed in only half of the cells with an amplitude that was ~7% of the wild-type current. This argues against misfolding and mistrafficking as a primary mechanism in the loss of function for R823W.

To further examine whether the CNBD mutant proteins are expressed on the plasma membrane, we visualized the location of HERG protein in living cells using N-terminal GFP fusion proteins for both wild-type and mutant HERG. GFP-tagged wild-type HERG produces K⁺ current similar to untagged wild-type HERG (data not shown). GFP-tagged β-adrenergic receptor (β-AR) was used as a control for plasma membrane location (30). CNBD mutations exhibited a similar fluorescence pattern as wild-type HERG and β-AR (Fig. 4). As frequently seen with transient forced expression, a signal was seen in endoplasmic reticulum-Golgi locations in all cells in addition to cell surface locations. These results confirm that defective biosynthetic processing and protein transport are not the major causes of the channel dysfunction in CNBD mutations.

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Recent reports have shown that loss of function associated with misfolded and mistrafficked mutant HERG may be rescued by incubation at low temperatures (24, 27, 28). We examined the effect of decreasing culture temperature on the expression of CNBD mutations. Previous work demonstrated that HERG existed as two bands on SDS-PAGE analysis when grown at 30 °C. No enhancement of mutant protein abundance occurred in cells that were grown at the lower temperature. Moreover, we were not able to detect any HERG current in CHO cells transfected with the V822M or ΔNBD mutants after incubation at 30 °C for 36–72 h. The R823W mutant, however, occasionally produced a very small amount of current when the incubation temperature was decreased to 27–30 °C for 24–48 h. Among 12 examined cells, 6 exhibited small HERG currents (Fig. 3d). The average tail current density for wild-type and R823W at 30 °C was 117.6 ± 14.5 pA/pF and 8.25 ± 2.7 pA/pF, respectively (p < 0.01). Despite comparable protein abundance, low temperatures resulted in a current that was expressed in only half of the cells with an amplitude that was ~7% of the wild-type current. This argues against misfolding and mistrafficking as a primary mechanism in the loss of function for R823W.

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Co-expression of CNBD Mutants with Wild-type HERG Produces Functional Heterotetrameric Channels—To assess the interactions between wild-type and CNBD mutant proteins, we co-expressed equal amounts of wild-type and mutant HERG in CHO cells and performed whole cell patch clamp recordings. Compared with wild-type HERG homotetramer, co-expression of wild-type with mutant HERG did not significantly alter current amplitudes (Table I and Fig. 5a). Alterations in \( V_{1/2} \) observed in currents from cells co-expressing wild-type HERG and CNBD mutants are evidence that support heterotetrameric channel assembly (Table I). When cAMP was applied during whole cell patch clamp measurements, the degree of current amplitude suppression seen in cells transfected with wild-type HERG alone was comparable with cells expressing

**Fig. 3.** **Protein expression of WT and CNBD mutant HERG.** a. Western blot analysis of whole cell lysates. Cells were harvested 24 h after transfection. GFP was used as a transfection efficiency indicator and a negative control. b. Western blot analysis of membrane (left panels) and cytosolic proteins (right panels). Both wild-type and CNBD mutant HERG are enriched in the plasma membrane fraction. As a control for transfection efficiency Myc-tagged connexin43 was co-transfected and appears in the lower left gel panel. c. effect of temperature on the expression and maturation of wild-type and CNBD mutations. 16 h after transfection, HEK293 cells were grown for another 36 h either at 37 °C or 30 °C. Wild-type HERG and CNBD mutations showed a mature glycosylated upper protein band around 155 kDa. d, reducing temperature to \( \approx 30 °C \) can partially rescue mutant R823W function. Upper panel shows examples of whole cell currents elicited by depolarizing steps from cells transfected with wild-type HERG (left) or R823W (right) after 36 h at 27 °C. Lower panel shows summary data of the tail current density of R823W HERG grown at \( \approx 30 °C \) was \( \sim 7\% \) of wild-type HERG.

**Fig. 4.** **Localization of WT and CNBD mutant HERG in living cells by GFP fluorescence.** CHO cells were transiently transfected with cDNA of N-terminal-tagged GFP wild-type and CNBD mutants for 48 h. Pairs of images for each cDNA show phase contrast (left panel) and GFP fluorescence images of the same cell (right panel). GFP-\( \beta \)-AR was used as a positive control for plasma membrane fluorescence. Wild-type and CNBD mutants showed a similar fluorescent location to that of GFP-\( \beta \)-AR.
both wild-type and CNBD mutants (Fig. 5c). This is consistent with our finding that cAMP-dependent current suppression is because of PKA-mediated phosphorylation of HERG (12).

Association of KCNE2 Uncovers an LQT2 Phenotype for CNBD HERG Mutants—The current belief is that HERG is complexed to small subunits of the KCNE family to form native I_{Kr} channels in vivo (4, 5). Furthermore, we reported that both KCNE1 and KCNE2 altered the effects of cAMP regulation of the HERG channel (12). To examine the effects of CNBD mutations on I_{Kr}, we co-expressed wild-type HERG and CNBD mutants with KCNE2. KCNE2 failed to induce currents from the functionally defective CNBD mutants in the absence of co-expressed wild-type HERG. The presence of KCNE2, however, did confer a suppressive effect on CNBD mutants when co-expressed with wild-type HERG. The exception to this was the R823Q mutant that can sustain current as a homotetramer (Table I and Fig. 5b). Compared with expression of wild type alone, current density for co-transfection with V822M, R823W, and ΔNBD were decreased 35.3%, 49.6, and 44.6%, respectively. Whereas this is a significant suppression, it does not represent a completely dominant-negative effect as seen with several other LQT2 mutants (24, 31). To exclude the possibility that co-expression KCNE2 with wild-type and mutant HERG would lower the expression of channel protein, we examined the abundance of total HERG protein by immunoblot. As seen in Fig. 5c, the total HERG protein abundance was comparable in the wild-type alone and mutant co-expression groups when KCNE2 was present. Assuming random assembly of an equal amount of wild-type and mutant subunits a binomial distribution would predict that a completely dominant mutation should reduce the current amplitude by >90% whereas the CNBD mutants range from ~35–50% current reduction. The cAMP effects on wild-type and mutant heteromultimers are altered when KCNE2 is co-expressed (Table I and Fig. 5b). In cells expressing WT-HERG-KCNE2, cAMP induced a leftward shift in voltage-dependent activation and did not significantly alter current amplitudes, which is consistent with our previous report (12). Addition of cAMP, however, causes an augmentation of current density for WT-V822M-KCNE2 channel and a suppression of the others. The V_{1/2} for WT-CNBD mutant-KCNE2 were all shifted to more negative voltages by cAMP.

Table I

| HERG channel type | V_{1/2} (mV) | ΔV_{1/2} cAMP | slope | Peak Current Density | Δ Density cAMP | Δ% | n |
|-------------------|-------------|---------------|-------|---------------------|---------------|-----|---|
| WT                | 2.86        | −0.47         | 9.33  | 0.47                | 31.51 ± 3.87  | −5.52 ± 1.73 | −17.5 | 20 |
| WT + V822M        | −6.23       | −2.42         | 9.36  | −0.17               | 27.55 ± 3.09  | −3.99 ± 1.92 | −14.5 | 14 |
| WT + R823Q        | 8.05        | −3.44         | 9.31  | −0.39               | 45.06 ± 6.48  | −12.37 ± 2.86 | −27.5 | 9  |
| WT + R823W        | 5.43        | −2.25         | 9.32  | −0.39               | 30.14 ± 6.22  | −3.42 ± 1.94 | −11.3 | 9  |
| WT + ΔNBD         | 13.65       | −4.51         | 9.71  | 0.32                | 37.55 ± 6.07  | −6.29 ± 3.14 | −16.8 | 5  |
| WT + KCNE2        | −1.2        | −3.6          | 10.4  | −0.7                | 75.39 ± 11.16 | −1.80 ± 5.97 | −2.4  | 7  |
| WT + KCNE2 + V822M| 4.9         | −5.05         | 11.1  | −0.8                | 48.77 ± 5.59  | 15.79 ± 5.31 | 32.4  | 8  |
| WT + KCNE2 + R823Q| 2.2         | −2.10         | 10.2  | 0.3                 | 76.89 ± 10.40 | −15.47 ± 7.05 | −20.1 | 6  |
| WT + KCNE2 + R823W| −5.68       | −6.13         | 12.51 | 0.28                | 37.96 ± 6.03  | −6.75 ± 4.52 | −17.8 | 6  |
| WT + KCNE2 + ΔNBD | −8.57       | −1.89         | 9.08  | 1.03                | 41.76 ± 6.61  | −7.69 ± 3.07 | −18.4 | 6  |

cAMP Binding Capacity of Wild-type HERG Protein and CNBD Mutations—Our previous work demonstrated that cAMP binds to HERG protein with a dissociation constant of ~40 μM (12). We measured the specific[^3H]cAMP binding of wild-type HERG and the CNBD mutants in the presence and absence of excess unlabeled cAMP. As shown in Fig. 7, in the absence of 400 μM unlabeled cAMP, only the wild-type HERG protein demonstrated a significant decrease in cAMP binding (p < 0.05), indicating a specific affinity for cAMP. The LQT2 mutant V822M and CNBD deletion mutant ANBD did not demonstrate specific binding to cAMP. The binding capacity for R823Q and R823W were significantly reduced compared with wild-type HERG. The loss of specific[^3H]cAMP binding capacity of HERG CNBD mutants suggests that the ability to bind cAMP is essential for normal channel function.
In this study we have examined the function of CNBD in HERG K⁺ channels in a heterologous mammalian expression system. We selectively introduced mutations at the key sites in the cyclic nucleotide binding domain, which we predicted would disrupt cAMP binding. With the exclusion of R823Q, we found that the CNBD mutants do not form functional channels as homotetramers despite normal protein expression. Immuno-blot analyses and microscopy of GFP fusion proteins demonstrated that CNBD mutant HERG surface expression was similar to wild-type HERG at physiological temperature. Although reduced incubation temperatures have been shown to rescue several other LQT2 mutants (24, 28), this failed to restore function to our CNBD mutants. Thus, defective protein transport to the plasma membrane does not appear to be the major reason for the functional defect in CNBD mutants. Furthermore, we show that CNBD mutant proteins have a decreased or absent capability to bind cAMP when compared with wild-type HERG. This suggests that the capacity to bind cAMP may be essential for normal HERG channel function.

Although CNBD mutant homotetramers did not express voltage-gated K⁺ currents, when co-expressed with wild-type HERG, functional heterotetrameric channels were detected. Unlike several other LQT2 mutations, the CNBD mutants that we examined demonstrated no dominant-negative suppression of wild-type current. This finding is consistent with previous reports of other C-terminal mutations (26, 28). The resulting current density values in our experiments co-expressing V822M with wild-type HERG at varying molar ratios is best fit to a model where mutant subunits are entirely non-dominant. We speculate that only one subunit capable of binding cAMP is needed for full channel function. Whether cAMP must actually bind to the channel for it to be active, however, is still not known.

Our model of a single cAMP binding subunit resulting in a functional channel has similarities to the activation of PKA and cyclic nucleotide-gated channels. The regulatory subunit of PKA has two cAMP binding sites, and mutational analysis demonstrates that activation of type I PKA requires only one of the cAMP binding sites to be capable of binding cAMP for preservation of function (14). Cyclic nucleotide-gated ion channels also contain a CNBD at the C terminus, analogous to HERG. Binding of a cyclic nucleotide to a single subunit is sufficient to produce significant activation of the channel. All four channel subunits, however, must bind to cyclic nucleotide for full current activation (32).

How the CNBD affects channel activity is still unknown. One possibility is that CNBD binding of cAMP is required for activation of the channel through a conformational change in the channel protein. Kupershmidt et al. (33) reported that upon expressing a recombinant HERG that entirely lacked the CNBD, a Ikr-like current was produced. Deletion of the entire CNBD however, is more likely to cause ancillary structural changes and channel perturbations in addition to loss of cAMP binding compared with point mutations that leave the CNBD intact but unable to bind cAMP. Liu et al. (32) have proposed a model where the CNBDs within a tetramer of cyclic nucleotide-gated channels act as ligand-bound dimers. Such a situation seems unlikely for HERG given the absence of dominant current suppression when CNBD mutants were co-expressed with wild-type HERG. For HERG CNBD to act as ligand-bound dimers we would have expected that increasing ratios of V822M/wild-type HERG would have suppressed the current in a fashion consistent with a 2-fold stoichiometry.

Another role that CNBD may play is to modulate HERG

![Functional co-expression of CNBD mutants and wild-type HERG. a, summary tail current density data from cells expressing wild-type HERG (WT) or wild-type HERG with CNBD mutants in a 1:1 molar ratio. Open bars represent the current amplitude at baseline and filled bars show the amplitude after cAMP treatment. b, similar to a, but for cells also co-expressing KCNE2 plasmid. *, p ≤ 0.05 by t test versus WT before treatment with cAMP. c, immunoblot analysis of HERG and KCNE2 protein expression. Cells were co-transfected with 1 μg of untagged wild-type HERG cDNA, 1 μg of Myc-tagged wild-type or CNBD mutant HERG cDNA and 1 μg of HA-tagged KCNE2 cDNA. The membrane was probed with HERG-specific polyclonal antiserum to demonstrate the total HERG protein abundance (top gel). Re-probing with anti-Myc antibody shows the expression of the Myc-tagged subunits (middle gel) and re-probing with anti-HA antibody shows the expression of KCNE2 (bottom gel).]
current by interacting with KCNE2. The interaction between HERG and a small integral membrane subunit such as KCNE2 can alter HERG channel current to more resemble native IKr (5). Moreover, we have shown that the co-expression of either minK or KCNE2 can significantly alter the cAMP-dependent response of HERG activity to favor direct effects as opposed to PKA-mediated effects (12). Although co-expression of CNBD mutant with WT HERG does not suppress current in a dominant-negative fashion, the addition of KCNE2 results in a mutant-dependent decrease in peak current density of about 40%. These results support a role for the CNBD of HERG in KCNE2 control of IKr. Clearly, further experiments are necessary to define the functional and structural interaction between the CNBD and KCNE2 in modulation of channel activity. Furthermore, it now appears that KCNE2 may play a pivotal role in mechanisms that create the LQTS phenotype for some LQT2 mutations. That the CNBD mutants were only partially dominant over wild-type function may be consistent with reports of another CNBD LQT2 mutant (26), and the observation that the clinical presentations of C-terminal mutations are less severe than those involving transmembrane and pore regions of HERG (7). Our present data however, suggest that association of KCNE2 with the HERG channel uncovers the dominant phenotype of CNBD LQT2 mutants. The degree of suppression we observed with differing ratios of mutant and wild-type HERG are best explained by multiple channel populations, some complexed with KCNE2 and some not. The stoichiometry of the KCNEs complexed to their respective α-subunits is not absolutely known; therefore, our results may alternatively represent variable degrees of mutant suppression resulting from different combinations of subunits.

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