PKA phosphorylation of HERG protein regulates the rate of channel synthesis

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Submitted 1 December 2008; accepted in final form 18 February 2009

Chen J, Sroubek J, Krishnan Y, Li Y, Bian J, McDonald TV. PKA phosphorylation of HERG protein regulates the rate of channel synthesis. Am J Physiol Heart Circ Physiol 296: H1244–H1254, 2009. First published February 20, 2009; doi:10.1152/ajpheart.01252.2008.—Acute changes in cAMP and protein kinase A (PKA) signaling can regulate ion channel protein activities such as gating. Effects on channels due to chronic PKA signaling, as in stress or disease states, are less understood. We examined the effects of prolonged PKA activity on the human ether-a-go-go-related gene (HERG) K+ channel in stably transfected human embryonic kidney (HEK)293 cells. Sustained elevation of cAMP by either chlorophenylthiol (CPT)-cAMP or forskolin increased the HERG channel protein abundance two- to fourfold within 24 h, with measurable difference as early as 4 h. The CAMP-induced augmentation was not due to changes in transcription and was specific for HERG compared with other cardiac K+ channels (Kv1.4, Kv1.5, Kir2.1, and KvLQT1). PKA activity was necessary for the effect on HERG protein and did not involve other cAMP signaling pathways. Direct PKA phosphorylation of the HERG protein was responsible for the CAMP-induced augmentation. Enhanced abundance of HERG protein was detected in endoplasmic reticulum-enriched, Golgi, and plasma membrane without significant changes in trafficking rates or patterns. An increase in the K+ current density carried by the HERG channel was also observed, but with a delay, suggesting that traffic to the surface is rate-limiting traffic. Acceleration of the HERG protein synthesis rate was the primary factor in the CAMP/PKA effect with lesser effects on protein stability. These results provide evidence for a novel mechanism whereby phosphorylation of a nascent protein dictates its rate of synthesis, resetting its steady-state abundance.

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myocytes. Cell suspension was filtered through a 250-μm mesh screen to separate myocytes from larger cardiac tissue and debris. The cells were washed three times in Ca²⁺-Tyrode’s and collected by centrifugation at 100 rpm for 1 min. Myocytes were resuspended in Ca²⁺-Tyrode’s solution and plated onto laminin-coated dishes. Cells were kept at 35–37°C in Tyrode’s solution for treatment with CPT-cAMP.

**Immunoblot, immunoprecipitation, and immunofluorescence.** Harvesting of cellular proteins, immunoprecipitation, and immunoblots was performed as previously described (22, 31). Immunoblot detection was performed by either chemiluminescent secondary antibody visualization (with multiple exposures to ensure linearity of signal) or fluorescently tagged secondary antibodies visualized with Odyssey infrared imaging system (LI-COR Biotechnology). Identical results were obtained with either system. All gels in the figures are representative of 2–6 independent experiments. Immunofluorescence staining of HERG, calnexin, and Golgi marker (GM130) was performed after the fixation of cells in 4% paraformaldehyde and permeabilization of HERG, calnexin, and Golgi marker (GM130) was performed to reduce fluorescence interference from beyond the focal plane.

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**Pulse-chase and metabolic labeling.** Cell proteins were metabolically labeled with 35S-cysteine/methionine (Trans-label; ICN) for pulse-chase studies as previously described (23). To examine the rate of HERG synthesis, cells were incubated in cysteine/methionine-free media for 1 h followed by the fresh RPMI 1640 medium containing 1,600 μCi/ml 35S-cysteine/methionine for various intervals (5, 20, 40, and 60 min). Labeling was halted by the addition of ice-cold detergent lysis buffer. Specific labeling of HERG was compared with both total cellular protein uptake of 35S and labeling of the housekeeping protein tubulin.

To evaluate the intracellular phosphorylation of ion channels, we used metabolic labeling of cells with 100 μCi/ml 32P-orthophosphate (Amersham) in phosphate-deficient media followed by stimulation with CPT-cAMP. Cells were subsequently lysed with ice-cold NDET buffer (150 mM NaCl, 1% Nonidet P-40, 25 mM Tris (pH 7.5), and 5 mM EDTA) and protease inhibitor cocktail (Roche) and centrifuged at 10,000 g for 10 min to remove nuclei and insoluble material. Lysates were precleared with protein-G-agarose (Pierce) and immunoprecipitated with anti-myc antibody, followed by protein-G-agarose. Precipitates were washed with cold NDET, separated by SDS-PAGE, and visualized by autoradiography and immunoblotting.

**Electrophysiology.** K⁺ currents conducted by HERG channels were measured using the whole cell configuration of the patch clamp as previously described (23). Internal pipette solution was composed of (in mM) 120 KCl, 2 MgCl₂, 0.5 CaCl₂, 5 EGTA, 4 ATP-Mg₂⁺, and 10 HEPES (pH 7.2; osmolality, 280 ± 10 mosm/l). External solution consisted of (in mM) 150 NaCl, 1.8 CaCl₂, 4 KC1, 1 MgCl₂, 5 glucose, and 10 HEPES (pH 7.4; osmolality, 320 ± 10 mosm). Whole cell capacitance was recorded (generally 10–30 pF) and was compensated by analog circuitry of the amplifier (Axopatch 200B; Axon Instruments). The holding potential was −80 mV, and currents were activated with depolarizing steps for 3.5 s followed by a repolarizing step to −40 mV (for 0.5 s) and then to −120 mV (0.25 s). Data acquisition and analysis were performed with pClamp 9 (Axon Instruments) and Origin 6.1 (Microcal) software.

**Real-time PCR.** Total RNA was obtained from HEK-HERG cells with and without CPT-cAMP using TRIZol (Invitrogen) and digestion with DNAse I. First-strand cDNA synthesis was performed with Superscript (Invitrogen). We then used the Mx3000P Real-Time PCR System with the Brilliant SYBR Green qPCR kit (Stratagene) to quantify HERG mRNA (GAPDH was used for normalization). Negative controls consisted of TRIZol- and DNAse I-digested cell samples omitting the reverse transcriptase. The HERG forward primer was 5'-TCAACCTGCAGATCCACCATG-3', and the reverse primer was 5’-CTGCGTCGCTCCGTCCTTT-3’. The PCR program is 95°C for 10 min, then 95°C for 30 s, 60°C for 30 s, and 72°C for 60 s at 40 cycles.

**Subcellular fractionation.** We followed the method of Hurt et al. (20) with minor adjustments. In brief, for each experiment one 15-cm dish of near-confluent HEK-HERG cells was washed twice with PBS; the cells were then exposed to 0.5-ml hypotonic buffer of 20 mM HEPES (pH 7.4), 2 mM EDTA, 2 mM EGTA, 6 mM MgCl₂, and protease inhibitors (Roche Pharmaceuticals) and carefully scraped off the plate in sheets. The cell suspension was subsequently homogenized by 3–10 strokes in a tight-fitting Dounce homogenizer and immediately centrifuged at 1,000 g for 5 min at 4°C. The supernatant was supplemented with 2.0 M sucrose to achieve a 0.2 M final sucrose concentration. A discontinuous sucrose gradient was made by overlaying the following sucrose concentrations made into the hypotonic buffer of 2.0 M (0.4 ml), 1.5 M (0.75 ml), 1.35 M (0.75 ml), 1.2 M (0.75 ml), 0.9 M (0.5 ml), and 0.5 M (0.5 ml). The cell lysate was loaded on top of this gradient and subjected to a 32,000 g centrifugation for 16 h in Sw60Ti rotor (Beckman Instruments). Thereafter, one 1.0-ml and seven 0.5-ml fractions were collected from the top and subjected to SDS-PAGE and immunoblot analysis, using rabbit-anti-HERG H175 1:200 (Santa Cruz), goat-anti-calnexin C20 1:200 (Santa Cruz), and mouse-anti-Na/K-ATPase 1:5,000 (AbCam) antibodies.

**RESULTS**

**Sustained elevation of cAMP increases HERG protein abundance.** To determine whether prolonged elevation of cAMP affected the HERG channel, we administered the membrane-permeable analog CPT-cAMP to HEK293 cells that stably express wild-type HERG-myc under control of a cyclo-megavir (CMV) promoter (HEK-HERG). Subsequent anti-myc immunoblots showed an increase in the abundance of HERG protein (Fig. 1, A and B). The increase was both time and dose dependent with a discernable increase by 4 h and with as little as 10⁻⁹ M CPT-cAMP. The well-described HERG doublet at 135 and 155 kDa represent the newer core-glycosylated and mature fully-glycosylated species, respectively (53). Both species of HERG increased as well as slower migrating immunoreactivity that represents higher order oligomeric channel complexes (Fig. 1, A and B). To control for potential effects of the myc-tag, we examined HEK cells transfected with either untagged HERG or 7X-histidine-tagged HERG. In both cases CPT-cAMP enhanced the HERG protein abundance in a comparable fashion (supplementary figure; all supplemental figures can be found with the online version of this article).

CPT-cAMP can directly interact with cAMP-binding proteins (such as PKA) and can increase endogenous concentration of cAMP by inhibiting phosphodiesterases (PDE), but its effects on endogenous cellular cAMP concentration are unpredictable due to possible PKA feedback on PDEs (8, 9). To determine whether endogenously generated cAMP had similar effects on HERG protein abundance, we exposed HERG-expressing cells to forskolin (an adenyl cyclase activator) or 3-isobutyl-1-methyl xanthine (IBMX; an inhibitor of PDE). Forskolin (between 1 μM and 1 mM) produced an increase comparable with CPT-cAMP in HERG protein abundance as did IBMX (but to a lesser extent; Fig. 1C). Likewise, in HEK cells expressing both HERG and β₁-adrenergic receptor, iso-
proterenol administration enhanced the HERG abundance, an effect that was further enhanced by IBMX (Fig. 1D).

To determine whether ether-a-go-go-related gene (ERG) channels in native cardiac cells respond in a similar fashion to cAMP elevation, we treated isolated rabbit cardiomyocytes in culture with CPT-cAMP. At a concentration of 20 μM, CPT-cAMP resulted in an increase of ERG protein assayed by immunoblot of 25–40% within 4–8 h (Fig. 1E).

cAMP-dependent enhancement of HERG is unique among cardiac K⁺ channels. To investigate whether the cAMP-induced enhancement of HERG protein is specific, we generated HEK293 cell lines that stably express different myc-tagged cardiac K⁺ channel α-subunits (Kv1.4-myc, Kv1.5-myc, Kir2.1-myc, and KCNQ1-myc) under control of the same CMV promoter. Cells were exposed to 0.5–50 μM CPT-cAMP, concentrations that resulted in the obvious enhancement in HERG after 24 h. Minimal changes in the abundance of Kir2.1, Kv1.4, Kv1.5, or KCNQ1 were seen with CPT-cAMP (Fig. 2A). To determine whether CPT-cAMP treatment resulted in channel phosphorylation of each of these channels, we metabolically labeled the cells with 32P-orthophosphate in a phosphate-deficient media followed by treatment of the cells with CPT-cAMP for 30 min. Subsequent purification of the channel proteins indicated that CPT-cAMP did enhance the phosphorylation of Kv1.5 and Kir2.1 to a similar extent as HERG (Fig. 2B). CPT-cAMP did not increase the phosphorylation of KCNQ1 as much as the other channels (~5% to 6%), which may represent the lack of coupling to the PKA-anchoring protein Yotiao (33). There was, however, considerable basal phosphorylation of the KCNQ1 channels, which may also represent constitutive phosphorylation in this system with no further PKA sites available. These results indicate that the cAMP-dependent increase in protein abundance was specific to HERG among the K⁺ channels examined. Furthermore, these results suggest that the effect on HERG is not due to enhanced transcription since each of the K⁺ channel cDNAs was driven by the same CMV promoter (pCI-Neo; Promega). This was confirmed by measurement of HERG mRNA by real-time PCR before and after CPT-cAMP treatment and normalized to GAPDH mRNA [50 mM cAMP-treated cells contained 70 ± 54% HERG mRNA compared with control cells; n = 4; P = 0.34, not significant (NS)].

Sustained elevation of cAMP increases HERG current density. To determine whether the cAMP-dependent enhancement of HERG protein resulted in a concomitant increase in functional current, we used the whole cell configuration of patch clamp. We examined cells in paired experiments (control vs. CPT-cAMP treatment) at two durations of treatment: 24 and 72 h (Fig. 3). To avoid the cAMP/PKA-dependent effects on channel gating (11, 46), we washed out the CPT-cAMP for 1 h.
before voltage-clamp recording. If residual channel-gating effects of PKA phosphorylation remain despite the CPT-cAMP washout, the results would be skewed away from observing an increase in current density. We compared the peak outward tail currents at +40 mV following a 3.5-s activating depolarization step to +60 mV. After 24 h of treatment with CPT-cAMP, cells exhibited a modest, but not statistically significant, enhancement in the current density of 11.3 ± 3.2% (33.6 ± 2.3 pA/pF for control cells and 37.9 ± 3.6 pA/pF with CPT cAMP; n = 11; NS; Fig. 3B). After 72 h of treatment with CPT-cAMP...
the current density, increased by $76 \pm 6.6\%$ (32.6 ± 2.1 pA/pF for control cells and $57.6 \pm 3.8$ pA/pF with CPT-cAMP; $n = 12; P < 0.001$; Fig. 3C). The delayed enhancement of current density relative to the time course of protein enhancement and plateau suggests either inefficient trafficking to the surface or that additional posttranslational modifications require more time than just changes in channel abundance. The voltage-dependence of activation was not significantly different between the groups (e.g., $V_h$ at 24 h, control $= -24.86 \pm 1.55$ mV and cAMP $= -26.97 \pm 1.38$ mV; at 72 h, control $= -21.25 \pm 0.14$ mV and cAMP $= -22.30 \pm 0.1$ mV; Fig. 3D).

Subcellular cAMP-dependent augmentation of HERG protein. To investigate whether the cAMP-dependent increase in both the immature and mature species of HERG was associated with changes in channel trafficking, we performed doublestaining immunofluorescence of control and of cells treated with CPT-cAMP using antibodies specific for GM130, a Golgi marker, or calnexin, an ER marker (Fig. 4A). After 24 h of CPT-cAMP treatment there was an increase in HERG signal in all synthesis and trafficking compartments as well as the surface. Colocalization, however, was most notably increased in the ER compartment, suggesting that the increase of HERG, after 24-h of CPT-cAMP, resulted from enhanced synthesis. That 24 h of elevated cAMP did not preferentially enhance HERG staining in Golgi or surface compared with ER suggests an absence of grossly perturbed HERG trafficking. To address this issue more precisely, we performed subcellular fractionation by velocity sedimentation of homogenized cell membranes on a discontinuous sucrose gradient. Gradient fractions were separated on SDS-PAGE and probed for HERG, Na/K-ATPase [plasma membrane marker (PM)], and calnexin (ER marker; Fig. 4B). When absolute HERG signal was quantified in ER- and PM-enriched fractions (Fig. 4C, left) both 24-h and 60-h time points showed an increase in channel protein. The earliest and greatest increase, however, was consistently in the ER fractions. The PM fraction HERG was more obvious at 60 h than at 24 h (Fig. 4C, insets). When we compared the quantity of HERG in ER or PM relative with the total amount in all fractions (Fig. 4C, right graphs), we observed that the relative amount of HERG in the ER was greater for the cAMP-treated cells. Thus it appears that the initial increase in channels occurs at the site of synthesis (the ER) with a delay.

Fig. 4. Subcellular compartmental increase in HERG with cAMP elevation. A: double-staining immunofluorescence analysis of HEK cells expressing HERG under control conditions, after treatment with CPT-cAMP or after treatment with H-89 (PKA inhibitor). Left panels show dual staining with the Golgi marker (GM)130, and right panels show dual staining with the endoplasmic reticulum-enriched (ER) marker calnexin. Merged panels show overlap and increased HERG signal in both ER and Golgi compartments. B: gradient fractions collected from velocity sedimentation of homogenized cells separated by SDS-PAGE show plasma membrane-enriched (PM) and ER patterns. Calnexin indicates the ER fractions, and Na/K-ATPase indicates the PM fraction. C: graphs at left show absolute amounts of HERG from each fraction at 24 and 60 h from the gels in B (note the different y-axis scales indicating greater accumulation of HERG at 60 h; comparable results were obtained in triplicate). Insets represent expanded scale values for plasma membrane HERG amounts showing a greater relative increase in HERG at 60 h compared with 24 h. Graphs at right show summary data (of 3 experiments) of relative amount of HERG in each fraction that is normalized to the total amount of HERG in all fractions.
until there is comparable enhancement at the surface, suggesting that trafficking is rate limiting but not specifically affected by cAMP. This result is consistent with the delay in cAMP-dependent HERG current augmentation (Fig. 3).

**Activation of PKA underlies the cAMP-dependent enhancement of HERG protein.** In addition to activating PKA to phosphorylate target proteins, cAMP can affect cellular processes by activating the guanine exchange factor Epacs (26). To distinguish between PKA and EPAC signaling as a mechanism for cAMP-dependent enhancement of HERG, we employed a combination of pharmacological methods. Double-staining immunofluorescence for HERG-myc with either Golgi or ER markers showed that H-89, a PKA inhibitor, produced a selective decrease in HERG protein abundance, whereas Sp-cAMP (which specifically activates PKA). 8CPT-2′ specifically activates the EPAC pathway) and Sp-cAMP examined the cAMP analogs 8CPT-2′-O-Me-cAMP (which specifically activates PKA). 8CPT-2′-O-Me-cAMP had no effect on HERG protein abundance, whereas Sp-cAMP produced an increase in HERG protein assayed by immunoblot (Fig. 5, A and B). These data support PKA as the mediator of the cAMP-dependent enhancement of HERG protein abundance. Furthermore, H-89 decreased both the basal HERG abundance and the cAMP-dependent enhancement of channel protein (Fig. 5C). These data do not, however, indicate the target(s) of PKA phosphorylation.

**cAMP/PKA signaling accelerates HERG protein synthesis.** The cAMP-dependent augmentation of HERG protein abundance could be due to a decreased rate of channel degradation (increased stability) or an enhanced rate of production. To distinguish between these two possible mechanisms, we performed metabolic labeling and pulse-chase experiments. Synthesis and stability of wild-type HERG-myc were determined by autoradiography of anti-myc immunoprecipitated 35S-labeled protein. Cells stably expressing HERG-myc were pretreated with 50 μM CPT-cAMP for 4 h before pulse-chase.

Once HERG is synthesized and assembled, its half-life in cultured cells is ~12 h (14, 34). CPT-cAMP treatment resulted in an increase in the half-life from ~13 h for control to ~16 h (Fig. 6, A and B). At the beginning of the chase period (time 0) and in the first 2 h, the predominant species is the immature core glycosylated form. After 8 h of chase the signal shifts almost entirely to the higher molecular weight mature form, a pattern not significantly different in control or CPT-cAMP-treated cells.

The increase in HERG stability in response to cAMP was relatively modest compared with the degree of accumulation we consistently saw over 24 h. We next examined the rate of channel synthesis as measured by 35S-cysteine/methionine incorporation into HERG (relative to that of total cellular proteins and tubulin, whose abundance was not altered by cAMP). The rate of 35S incorporation into HERG over a period of 60 min was approximately doubled in cells that had been pretreated with CPT-cAMP (Fig. 6, C and D). We did not observe comparable changes in 35S accumulation in tubulin. There is a doubling of HERG synthesis rate within the first 60 min of cAMP but immunoblots take longer to detect changes in HERG abundance (Fig. 1A), which reflect the long half-life of the channel protein such that newly synthesized channels comprise a relatively smaller portion of the total population of channels. Thus the cAMP-dependent augmentation of HERG protein abundance is, primarily, due to an acceleration of synthesis with a lesser contribution from the enhanced stabilization of channel protein.

**PKA phosphorylation of HERG channel enhances its abundance.** HERG contains four sites that can be phosphorylated by PKA (S283, S890, T895, and S1137), and the acute functional effect of phosphorylation of these sites has been determined (11, 46). Additional PKA sites do not exist in HERG (11). To determine whether the direct phosphorylation of the HERG protein by PKA was required for the cAMP-dependent augmentation, we generated HEK293 cell lines that stably express the PKA site mutant Δ1234HERG where the acceptor serines and threonine were mutated to a nonphosphate accepting alanine (S283A, S890A, T895A, and S1137A). When cells expressing Δ1234HERG were treated with CPT-cAMP for 24 h, the cAMP-dependent augmentation of HERG protein was consistently reduced to 5–10% compared with the several fold increase seen with wild-type protein (Fig. 7A). When we examined the rate of 35S incorporation into the Δ1234HERG mutant, CPT-cAMP resulted in minimal increase in synthesis (Fig. 7B). These results point to direct phosphorylation of the
channel as the responsible mechanism for cAMP/PKA-dependent enhancement of HERG abundance.

**cAMP/PKA activity enhances the rate of HERG protein translation.** Enhancement of the synthesis of a protein may be controlled at the gene transcription level, enhanced mRNA stability, polypeptide translation rate, or shunting of nascent protein products to the proteasome for degradation. That there was no increase in HERG mRNA with CPT-cAMP treatment argues against the first two mechanisms. To distinguish between the other two we examined the effects of translation arrest during CPT-cAMP treatment. Cycloheximide, which blocks the translocation process during protein synthesis, sup-

Fig. 6. cAMP effects on HERG protein stability and rate of synthesis. **A:** autoradiograph of metabolically labeled HERG in pulse-chase. A modest increase in the stability of HERG is observed with CPT-cAMP treatment. **B:** graphical representation of densitometry from pulse-chase analysis. **C:** incorporation of 35S-methionine/cysteine in cells with or without CPT-cAMP treatment is seen by autoradiograph of immunoprecipitated HERG-myc. Bottom gels show the rate of incorporation of 35S into tubulin under conditions of control of CPT-cAMP treatment. **D:** graphical representation of incorporation of metabolic label show marked acceleration of synthesis with cAMP. Values are arbitrary densitometry units normalized to earliest signal detectable (n = 4). Bottom graphs show the rate of incorporation of 35S into tubulin with no significant difference between control and CPT-cAMP groups.

Fig. 7. PKA phosphorylation of the HERG channel is required for cAMP-dependent increase in protein. **A:** immunoblot analysis of cells stably expressing either wild-type HERG (WT) or with the Δ1234HERG mutant with all 4 PKA acceptor sites mutated to alanine (Δ1234). Cells were treated with 50 μM CPT-cAMP for 24 h. Right panel shows graphical summary of cAMP-dependent change in abundance of WT or Δ1234HERG in paired experiments (n = 5). **B:** 35S accumulation rates for Δ1234HERG are shown under control and CPT-cAMP treatment. Right panel shows graphical summary of 35S accumulation in Δ1234HERG.
pressed basal HERG abundance and at a concentration of 75 µg/ml prevented most of the cAMP-dependent increase (Fig. 8A). Tubulin has a very long half-life [48–50 h (6, 44)] as opposed to the half-life of HERG (Fig. 6). Tubulin is synthesized at a comparatively slow rate (Fig. 6C). Accordingly, we observed much smaller changes in the steady-state tubulin amount with cycloheximide over the duration of the experiment. Newly translated proteins may be shunted to the 26S proteasome for early degradation, even before the completion of synthesis (41, 43). If this process was reduced, the result would be an apparent increase in the synthesis rate of proteins. To examine this possibility we used two different proteasome inhibitors: lactacystin and MG-132. Both reagents slightly enhanced basal amounts of HERG protein; however, the cAMP responsiveness remained intact (Fig. 8B). Proteasome inhibition had no effect on either the concentration or cAMP effects of the Δ1234HERG mutant.

**Heat shock proteins and cAMP-dependent augmentation of HERG.** Ficker and coworkers (14) have clearly established that heat shock protein (HSP)90 and HSP70 interact with newly synthesized and assembling HERG protein and play an important role in both maturation of normal channels and degradation of misfolded HERG. To determine whether cAMP/PKA activation impacts the processing of HERG through alteration of HSP90, we treated cells with CPT-cAMP and geldanamycin (an inhibitor of HSP90) and assayed HERG-myc, HSP90, heat shock cognate (HSC)70, HSP70, and tubulin (Fig. 9). As previously described (14), geldanamycin reduced HERG protein maturation (as seen by the preferential reduction of the mature glycosylation form). Geldanamycin at 1 µg/ml completely abolished cAMP-dependent augmentation of HERG protein. At the lower concentration (0.1 µg/ml) of geldanamycin, cAMP responsiveness was retained. CPT-cAMP did not alter the abundance of HSP90, HSP70, or HSC70. Thus cAMP/ PKA did not appear to exert its effects on HERG by directly influencing the chaperones. The abundance of HSP70 was enhanced by treatment with geldanamycin, as previously described (54).

**DISCUSSION**

In the present study, we show that prolonged elevation of cAMP can substantially increase HERG protein abundance by a novel mechanism that involves PKA phosphorylation of the channel. This process leads to accelerated synthesis of HERG protein and enhanced current density. There was no evidence that other cAMP targets were involved in the process. The augmentation of channel abundance was specific to HERG in that comparable changes were not observed in the other cardiac potassium channels tested (Kv1.4, Kv1.5, Kir2.1, and KCNQ1). The increase in HERG channel abundance, however, was not the result of, nor accompanied by obvious changes in, HERG trafficking, but trafficking to the cell surface was a rate-limiting step in potassium conductance increase. The cAMP-dependent augmentation of HERG was due to stimulation of PKA phosphorylation of the channel with an ensuing enhancement of translation rate. This is the first example of PKA phosphorylation of a client protein altering its rate of translation. Regulation of ion channels by means of cAMP/...
PKA signaling is well established (30); however, studies have largely focused on short-term effects on channel gating. This applies as well to HERG where the cAMP/PKA pathway has been shown to acutely regulate channel function. The present study differs in that the effect of prolonged cAMP perturbation upon HERG channels was studied.

Prolonged cAMP signaling may impact various aspects of protein processing and function for several other channels. Chronic stimulation of Xenopus oocytes with the cAMP analog (Sp)-8-Br-cAMP increased current density with both increased abundance and cell surface expression of heterologously expressed Kv1.1 although the mechanism was not explored (29). Prolonged treatment (3–5 days) of serum-starved adrenal chromaffin cells with CPT-cAMP increased the current density of T type calcium channels via the EPAC pathway by an undefined mechanism (39). The inward rectifier potassium channel, Kir1.1, when phosphorylated by PKA generates an ER exit signal for regulated trafficking of channels to the cell surface (40). Dynamic surface targeting of the aquaporin channel AQ2 in response to vasopressin is mediated by PKA phosphorylation of the channel protein (38). Such a mechanism does not appear to play a role in the cAMP/PKA-dependent enhancement of HERG; to the contrary, exit from the ER appears to be a bottleneck for HERG channel trafficking that is rate-limiting despite the increased channel production. Synthesis and assembly of LQT2 mutant and even wild-type HERG is an inefficient process that appears to yield a considerable amount of misfolded product that never becomes functional channels (18, 19, 22, 52). Of the ~291 known LQT-causing mutations in HERG (http://www.fsmt/cardio.html), many, if not most, result in misfolding with ensuing mistrafficking and destruction via the proteasome (1, 45). Dominant negative HERG trafficking deficiency can decreased wild-type HERG abundance, increased its degradation, and retain wild-type HERG in ER (13, 17, 23).

Several molecular mechanisms have been implicated in the control of HERG processing. Perhaps the best described is the cytosolic chaperone HSP70 and HSP90, which have been clearly shown as essential for the maturation of HERG (14). Ficker and colleagues showed HSP70 and HSP90 transiently bind the nascent HERG and increase trafficking-defective HERG retention in ER, thereby impairing their involvement in cellular quality control. That geldanamycin completely inhibited the cAMP-mediated enhancement in our study suggests that the site of cAMP/PKA regulation is either at the HSP90 interaction or involves an upstream step in the biogenesis of HERG. If geldanamycin had failed to prevent the effect, the interpretation would have been that cAMP increases HERG by influencing steps after the HSP90 interaction. The role of HSP90 and its list of known clients in eukaryotic cells is more restricted than those of HSP/HSC70. HSP90 is thought to facilitate later folding and assembly activities under stress conditions or for inherently difficult proteins (4, 36, 50).

If nascent peptide chains exiting the ribosome have not achieved proper secondary structure, they may be at risk for aggregation, particularly if hydrophobic residues are exposed to the aqueous environment. Chaperones such as HSPs are important in preventing aggregation by binding to sites at risk until the new protein can properly assume its stable and ultimately functional conformation (5, 15). These events may occur in early steps of protein translation, folding, translocation, and degradation (7, 35, 51). Under normal cellular conditions, a considerable fraction of newly translated proteins is targeted for early and rapid degradation, with a half-life that is less than 10 min (41, 43). We speculate that a possible mechanism for our observation that cAMP/PKA enhances HERG translation is that phosphorylation of the newly forming protein promotes productive folding events, thereby facilitating further translation of complete product. Our observation that cAMP increased the phosphorylation of the immature form of HERG (thought to represent channels either in ER or early Golgi; Fig. 2B) supports this possibility. The precise constituents of such a complex that recruits PKA to the site of translation in the ER remain to be determined.

Phosphorylation of proteins has been shown to alter a wide variety of processes including enzymatic behavior, channel gating, subcellular trafficking, degradation, and protein-protein interactions. The data presented here, to our knowledge, may be the first demonstration of altered protein translation caused by phosphorylation. There is evidence that phosphorylation of tau has pathogenic properties in Alzheimer’s disease and that phosphorylation is a trigger for HSP70 binding in an attempt to target the cytosolic protein for degradation (27). Gao and Newton (16) have shown that PKC (along with Akt and PKA) interacted selectively with HSP70 when in the nonphosphorylated state with the effect that the proteins were stabilized, but without altered rates of synthesis or maturation (16).

Autonomic regulation of the heart includes transient up- and downregulation of sympathetic and parasympathetic stimulation that results in normal heart rate variability dictated by hemodynamic demands. Work by Kiehn, Thomas, and colleagues and our laboratory have established that the cAMP/PKA pathway is a regulator of HERG channel function acutely (10, 11, 22, 24, 46). The cAMP/PKA regulation of HERG described in these studies represents acute regulation as might be seen with normal, rapid changes in adrenergic stimulation. Chronic elevations in sympathetic stimulation (epinephrine and norepinephrine) frequently accompany a variety of heart diseases. These changes initially compensate for decreased cardiac function but eventually become counterproductive and contribute to pathophysiology. Downregulation of β-adrenergic receptors and secondary signaling via Gsx, adenyly cyclase, and cAMP/PKA signaling is a hallmark of these states (21, 32, 42, 48). Treatment with β-adrenergic blocking drugs has become the mainstay of therapy for many types of heart disease including ischemia, failure, hypertension, and hypertrophy. Clinical trials almost invariably show improved function and survival with β-blocker therapy. Some portion in the improved survival may be due to reduction of lethal ventricular arrhythmias (2, 28). The initial characterization of cAMP/PKA regulation of the abundance of a protein in the study presented here may indicate one of the ultimate cellular targets of sympathetic overstimulation, downregulation, and during β-adrenergic therapies.

**ACKNOWLEDGMENTS**

We thank Dr. Charles Rubin for thoughtful discussion of this work.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant HL-077326 to T. V. McDonald. Y. Li is a recipient of a postdoctoral fellowship from the Heritage Affiliate of the American Heart Association, and...
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