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AKAP proteins anchor cAMP-dependent protein kinase to KvLQT1/IsK channel complex

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Potet, Franck, John D. Scott, Raha Mohammad-Panah, Denis Escande, and Isabelle Baró. AKAP proteins anchor cAMP-dependent protein kinase to KvLQT1/IsK channel complex. *Am J Physiol Heart Circ Physiol* 280: H2038–H2045, 2001.—In cardiac myocytes, the slow component of the delayed rectifier K^+ current (I_{Ks}) is regulated by cAMP. Elevated cAMP increases I_{Ks} amplitude, slows its deactivation kinetics, and shifts its activation curve. At the molecular level, I_{Ks} channels are composed of KvLQT1/IsK complexes. In a variety of mammalian heterologous expression systems maintained at physiological temperature, we explored cAMP regulation of recombinant KvLQT1/IsK complexes. In these systems, KvLQT1/IsK complexes were totally insensitive to cAMP regulation. cAMP regulation was not restored by coexpression with the dominant negative isoform of KvLQT1 or with the cystic fibrosis transmembrane regulator. In contrast, coexpression of the neuronal A kinase anchoring protein (AKAP)79, a fragment of a cardiac AKAP (mAKAP), or cardiac AKAP15/18 restored cAMP regulation of KvLQT1/IsK complexes inasmuch as cAMP stimulation increased the I_{Ks} amplitude, increased its deactivation time constant, and negatively shifted its activation curve. However, in cells expressing an AKAP, the effects of cAMP stimulation on the I_{Ks} amplitude remained modest compared with those previously reported in cardiac myocytes. The effects of cAMP stimulation were fully prevented by including the Ht31 peptide (a global disruptor of protein kinase A anchoring) in the intracellular medium. We concluded that cAMP regulation of I_{Ks} requires protein kinase A anchoring by AKAPs, which therefore participate with the channel protein complex underlying I_{Ks}.

A kinase anchoring protein; *KCNQ1*; *KCNE1*; slow delayed rectifier potassium current

HORMONAL STIMULATION OF THE HEART involves the control of Ca^{2+} and K^+ currents. These events are triggered by a multistep pathway comprising receptor-mediated activation of adenylyl cyclase and the generation of the diffusable second messenger cAMP, resulting in the activation of protein kinase A (PKA) (25). In guinea pig ventricular myocytes, PKA stimulation produces an increase in the amplitude of the delayed rectifier K^+ current (27, 28, 32). PKA activation also promotes a negative shift in its activation curve and an alteration of its deactivation kinetics (27, 28). It was previously shown that the slow component of the delayed rectifier K^+ current (I_{Ks}) constitutes the K^+ current component sensitive to β-adrenergic stimulations (27). In the heart muscle, I_{Ks} is related to the activity of the KvLQT1 K^+ channel protein in tandem with its regulator, termed IsK (or minK) (2, 22). KvLQT1 channel proteins but not IsK possess a putative consensus phosphorylation site by PKA between residues 24 and 27. Expression of the human KvLQT1 proteins alone in *Xenopus* oocytes underlies a voltage-dependent K^+ current that is sensitive to PKA stimulation (31), whereas expression of IsK alone activates an endogenous KvLQT1-like protein that generates a cAMP-sensitive voltage-dependent K^+ current similar to I_{Ks} (13, 21, 26, 31).

In the present study, we investigated the regulation of the human recombinant KvLQT1/IsK complex in mammalian cell lines maintained at physiological temperature. Surprisingly, these expression systems lacked key element(s) to permit cAMP activation of the I_{Ks}. cAMP regulation of I_{Ks} was restored by coexpression of KvLQT1/IsK together with A kinase anchoring proteins (AKAPs). AKAPs comprise a group of proteins with a proposed role in mediating the attachment of type II PKA to subcellular structures (20). We concluded that AKAPs are a key element for regulation of I_{Ks} by cAMP and may be additional protein components of the I_{Ks} channel functional complex.

MATERIALS AND METHODS

Intranuclear injection of plasmids. COS-7 cells [American Type Culture Collection (ATTC); Manassas, VA] were cultured in Dulbecco's medium supplemented with 10% fetal calf serum and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin; all from GIBCO-BRL; Paisley, Scotland) at 37°C in a humidified incubator. Swiss 3T3 fibroblasts and HEK293 cells were also obtained from the ATTC. Cultured

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cells were microinjected into the nucleus with plasmids at *day 1* after plating. The protocol to microinject cultured cells using the Eppendorf ECET microinjector 5246 system has been previously reported (17). Briefly, plasmids were diluted in a buffer made of (in mmol/l) 40 NaCl, 50 HEPES, and 50 NaOH; pH 7.4 supplemented with 0.5% fluorescein isothiocyanate-dextran (150 kDa). Human cardiac KvLQT1 isoform 1, KvLQT1 isoform 2, and the cystic fibrosis transmembrane conductance regulator (CFTR) were subcloned into the mammalian expression vector pCI (Promega; Madison, VI) under the control of a cytomegalovirus enhancer/promoter. AKAP79, a fragment of cardiac AKAP (mAKAP), and AKAP15/18 were subcloned into the mammalian expression vector pCDNA3. Human IsK cDNA was subcloned into a pRC vector under the control of a cytomegalovirus promoter. CFTR cDNA was subcloned into a pCDNA3 plasmid. A green fluorescence protein pCI plasmid (a gift from Dr. Rainer Waldmann; Sophia-Antipolis, France) was used as an inert coplasmid to ensure that cells were always injected with a constant 15 μg/ml plasmid concentration.

Patch-clamp recordings. Whole cell currents were recorded as previously described (17). Cells were placed on the stage of an inverted microscope and continuously superfused with the standard extracellular solution. Patch pipettes with a tip resistance of 2.5–5 MΩ were electrically connected to a patch-clamp amplifier (Axopatch 200A, Axon Instruments; Foster City, CA). Stimulation, data recording, and analysis were performed through an analog-to-digital converter (Tecmar TM100 Labmaster, Scientific Solutions; Solon, OH) and Acquis1 software (Bio-Logic; Claix, France). A microperfusion system allowed local application and rapid change of the different experimental solutions warmed at 35°C. Current measurements were normalized using the cell capacitance. Patch-clamp measurements are presented as the means ± SE. Statistical significance of the observed effects was assessed by means of the paired *t*-test or two-way ANOVA when needed. A value of $P < 0.05$ was considered significant.

Solutions and drugs. The standard extracellular medium contained (in mmol/l) 145 NaCl, 4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, and 5 glucose; pH was adjusted to 7.4 with NaOH. The intracellular medium contained (in mmol/l) 145 potassium gluconate, 5 HEPES, 2 EGTA, 2 hemimagnesium gluconate (free Mg^{2+}: 0.1), and 2 K_2ATP; pH 7.2 with KOH, whereas the extracellular medium used to record K^+ currents contained (in mmol/l) 145 sodium gluconate, 4 potassium gluconate, 7 hemicalcium gluconate (free Ca^{2+}: 1), 4 hemimagnesium gluconate (free Mg^{2+}: 1), 5 HEPES, and 5 glucose; pH 7.4 with NaOH. Free activities were calculated using a software designed by G. L. Smith (University of Glasgow, Glasglow, UK). Intracellular cAMP was increased with a mixture made of 10 μmol/l forskolin plus 400 μmol/l 8-(4-chlorophenylthio)-cAMP (cpt-cAMP) (both from Sigma; St. Louis, MO). The anchoring inhibitor peptide derived from Ht31 was used at concentration of 1 μmol/l in the intracellular solution (3, 19).

RESULTS

Effects of cAMP on KvLQT1/IsK complex expressed in mammalian cells. With the objective of investigating the PKA-mediated regulation of recombinant I_{Ks} in a mammalian expression system, human KvLQT1 and IsK plasmids were injected into COS-7 cells. The whole cell configuration of the patch-clamp technique was used to assess the formation of functional channel complexes. Results obtained in cells injected with 2.5 μg/ml pCI-KvLQT1 and 2.5 μg/ml pRC-IsK are summarized in Fig. 1. As shown in Fig. 1, *A* and *B*, the I_{Ks} current elicited by a depolarizing step to +40 mV was

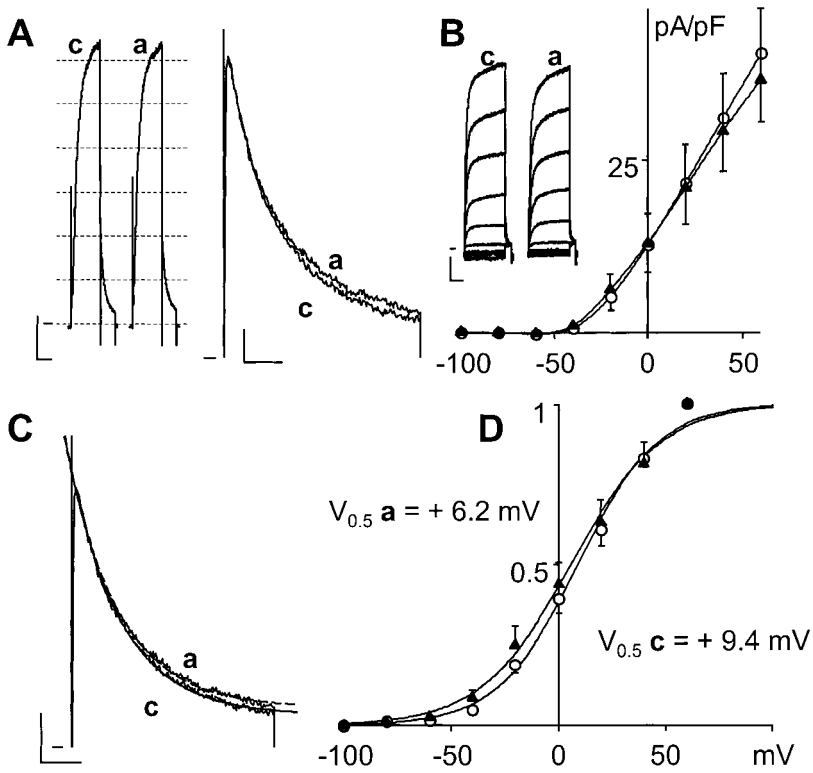

Fig. 1. Effect of cAMP on recombinant slow component of delayed rectifier K^+ current (I_{Ks}) in COS-7 cells recorded at physiological temperature. *A*, *left*: typical K^+ currents recorded in a cell injected with 2.5 μg/ml pCI-KvLQT1 isoform 1 and 2.5 μg/ml pRC-IsK in control conditions (*trace c*) and after cAMP stimulation (*trace a*). The membrane potential was stepped from −80 mV to +40 mV and then to −40 mV (vertical bar, 200 pA; horizontal bar, 500 ms). *Right*: magnified tail currents (vertical bar, 50 pA; horizontal bar, 100 ms). *B*: current-voltage (*I-V*) relation for I_{Ks} in control conditions (○) and under cAMP stimulation (▲; $n = 8$). *Inset*: superimposed current traces elicited by test potentials between −100 and +60 mV (increment, 20 mV; holding potential, −80 mV; vertical bar, 250 pA; horizontal bar, 1,000 ms). *C*: superimposed normalized tail currents before and after cAMP stimulation. Deactivation kinetics were fitted to a single exponential [*trace c*: solid line, deactivation time constant (τ_{deact}) = 103 ms; *trace a*: dashed line, τ_{deact} = 104 ms; vertical bar, 20% maximum current (I_{max}); horizontal bar, 100 ms]. *D*: activation curves for I_{Ks} before (○) and after (▲, $n = 5$) cAMP stimulation. $V_{0.5}$, half-activation potential.

totally insensitive to cAMP stimulation produced by adding 10 μmol/l forskolin plus 400 μmol/l cpt-cAMP to the extracellular medium. On average, the voltage-activated K^+ current measured during the depolarizing step to +40 mV was 27.5 ± 7.2 pA/pF in control versus 28.9 ± 7.6 pA/pF under cAMP stimulation [n = 8; P = not significant (NS)]. In the same expression system, we have previously shown that intracellular cAMP increases at least 10-fold in response to 10 μmol/l forskolin, thereby allowing PKA-mediated activation of CFTR channels (12). Therefore, the absence of response to forskolin plus cpt-cAMP of KvLQT1/IsK complexes was unlikely to be caused by low intracellular cAMP. Deactivating tail currents at −40 mV were fitted by a single-exponential decay, which was extrapolated to *time 0* so as to reliably measure tail current amplitude. As illustrated in Fig. 1, *A* and *C*, no difference was observed in either the tail current amplitude (9.6 ± 2.4 pA/pF in control vs. 9.5 ± 2.4 pA/pF with cAMP, respectively; n = 8) or deactivation kinetics [deactivation time constant (τ_{deact}) was 95.4 ± 7.1 ms in control vs. 101.7 ± 10.6 ms with cAMP, n = 6; Fig. 1*C*] in the absence or presence of cAMP stimulation. The voltage-dependence of the I_{Ks} was also investigated. Neither the current-voltage relation for I_{Ks} (Fig. 1*B*) nor its activation (Fig. 1*D*) were affected by increased cAMP: the half-activation potential ($V_{0.5}$) was 9.4 ± 3.4 mV in control conditions versus 6.2 ± 5.8 mV under cAMP stimulation (n = 5). Additional experiments were also carried out in other cell lines including HEK293 and 3T3 fibroblast cells. These experiments produced similar negative data as obtained in COS-7 cells (data not shown). Thus this first set of experiments demonstrated that KvLQT1/IsK channel complexes expressed in mammalian cells are insensitive to cAMP-mediated PKA activation. We next tested whether a regulating protein, absent in host mammalian cells, was involved in the sensitivity of KvLQT1/IsK complex to cAMP stimulation.

Effects of cAMP on I_{Ks} in the presence of regulating proteins. KvLQT1 isoform 2 is a *KCNQ1* gene alternative splice variant constitutively expressed in the adult human heart, which participates in the I_{KS} channel complex (4). Isoform 2 has an NH_2-terminal sequence 131 amino acids shorter than the cardiac KvLQT1 full-length isoform (isoform 1) (4) and thus lacks the consensus protein kinase phosphorylation site localized at amino acids 24–27 of isoform 1. In a previous work (4), we have shown that COS-7 cells expressing both KvLQT1 isoform 1 and isoform 2 in the presence of IsK exhibited a K^+ current with a reduced amplitude compared with cells expressing KvLQT1 isoform 1 plus IsK. We decided to test whether coexpression of isoform 2 as in cardiac myocytes interferes with protein kinase phosphorylation of isoform 1. As shown in Fig. 2*A*, cells injected with KvLQT1 isoform 1, KvLQT1 isoform 2, and IsK plasmids exhibited an I_{Ks} that was also insensitive to cAMP stimulation (n = 7). Varying the concentration of IsK or KvLQT1 isoform 2 plasmids in the injection medium did not affect cAMP regulation of I_{Ks}.

We (14) have previously shown that CFTR possesses properties of K^+ channel regulator in epithelial cells. In pancreatic epithelial cells, a K^+ current is elicited by cAMP stimulation in the presence of a functional CFTR expression but not in its absence (14). Because KvLQT1 has also an epithelial expression (5) and because CFTR is also expressed in heart muscle, we tested the hypothesis of CFTR-dependent KvLQT1 regulation by PKA. We investigated the effects of cAMP stimulation on I_{Ks} recorded in COS-7 cells expressing the KvLQT1 isoform 1/IsK channel plus CFTR. Typical results from these experiments are illustrated in Fig. 2*B*. Again, in seven different cells, we did not observed any significant change in the I_{Ks} amplitude in response to cAMP stimulation. In these experiments, we checked for expression of functional CFTR by making use of the 6-methoxy-*N*-(3-sulfopropyl) quinolinium assay (12). Recently, it has been shown that AKAPs mediate the

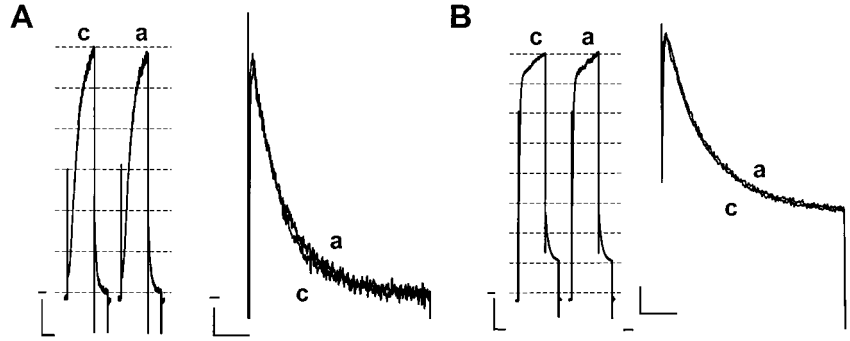

Fig. 2. Effect of cAMP on I_{Ks} in cells coexpressing KvLQT1 isoform 2 or the cystic fibrosis transmembrane conductance regulator (CFTR). *A*, *left*: K^+ current recorded in a COS-7 cell injected with 5 μg/ml pCI-KvLQT1 isoform 1, 5 μg/ml pRC-IsK, and 2 μg/ml pCI-KvLQT1 isoform 2 (same protocol and abbreviations as in Fig. 1*A*) in control conditions (*trace c*) and after cAMP (*trace a*) (vertical bar, 100 pA; horizontal bar, 500 ms). *Right*: magnified tail currents recorded under same experimental conditions (vertical bar, 50 pA; horizontal bar, 100 ms). *B*, *left*: K^+ currents recorded in a cell injected with 5 μg/ml pCI-KvLQT1 isoform 1, 5 μg/ml pRC-IsK, and 5 μg/ml pCDNA3-CFTR (same voltage protocol as in Fig. 1*A*). Vertical bar, 200 pA; horizontal bar, 500 ms. *Right*: vertical bar, 20 pA; horizontal bar, 100 ms.

effects of PKA on various ion channels (1, 7, 11, 19). We thus explored whether the absence of AKAPs in our expression system was responsible for the failure of the KvLQT1/IsK channels to respond to cAMP.

Effect of cAMP on I_{Ks} in the presence of AKAPs. COS-7 cells were coinjected with KvLQT1, IsK, and AKAP79 plasmids. In the absence of cAMP stimulation, the characteristics of I_{Ks} generated by the KvLQT1/IsK complex in the presence of AKAP79 were undistinguishable from those recorded in its absence. In cells expressing KvLQT1 plus IsK, the K^+ current amplitude at +40 mV was 25.9 ± 5.1 pA/pF (n = 12), i.e., not different from the value obtained in cells coexpressing AKAP79 (26.9 ± 5.8 pA/pF; n = 10). Other parameters of I_{Ks} were not altered by AKAP79 expression including activation and deactivation kinetics and voltage dependence. In contrast, in cells expressing AKAP79, the amplitude of the K^+ current related to KvLQT1/IsK expression was consistently enhanced by 10 μmol/l forskolin plus 400 μmol/l cpt-cAMP (Fig. 3, *A* and *B*). The tail current amplitude increased from 7.8 ± 2.6 to 8.6 ± 2.5 pA/pF (+15%, n = 6, $P < 0.01$) under cAMP stimulation. In addition, τ_{deact} also increased from 104.5 ± 16 to 122.9 ± 17 ms (n = 6, $P < 0.001$; Fig. 3*C*). Figure 3*D* illustrates the time course of I_{Ks} tail current amplitude and τ_{deact} changes as produced by cAMP enhancement. In native cardiac cells, phosphorylating agents shift the activation curve of I_{Ks} toward more negative potentials (28, 32). In accordance with this, in our cells, the I_{Ks} activation curve shifted toward more negative potentials under cAMP (Fig. 3*E*; $V_{0.5}$ was 12.9. ± 6.5 mV under control vs. 3.6 ± 5.7 mV with cAMP, n = 6, $P < 0.01$). Thus AKAP79 expression in COS-7 cells restores regulation by cAMP of the expressed KvLQT1/IsK channel.

Recently, it has been reported that mAKAP tethers PKA to multiple subcellular compartments in cardiac myocytes (16, 30). As with AKAP79, the characteristics of I_{Ks} generated by the KvLQT1/IsK complex in the absence of cAMP stimulation were not modified by mAKAP expression. In cells coexpressing mAKAP, the K^+ current amplitude at +40 mV was 21.3 ± 3.1 pA/pF (n = 18), i.e., not different from the K^+ current amplitude in cells not expressing mAKAP. As illustrated in Fig. 4, coexpression of mAKAP restored cAMP sensitivity of I_{Ks} inasmuch as cAMP stimulation increased the current amplitude, slowed the deactivation kinetics, and shifted to more negative potentials the $V_{0.5}$ from 13.9. ± 2.8 to 3.2 ± 3.3 mV (n = 10, $P < 0.001$). In a parallel study (9), we observed that AKAP15/18, another cardiac AKAP, was also capable of reconstituting cAMP regulation of the KvLQT1/IsK complex (Fig. 4*F*). Again, in the presence of AKAP15/18, the baseline characteristics of I_{Ks} were undistinguishable from those determined in its absence. In cells coexpressing AKAP15/18, the K^+ current amplitude at +40 mV was 30.6 ± 5.8 pA/pF (n = 12), i.e., not significantly different from the K^+ current amplitude in cells not expressing AKAP15/18. In AKAP15/18-expressing cells submitted to cAMP stimulation, the tail current amplitude increased from 9.4 ± 1.5 to 11.5 ± 1.6 pA/pF (+22%, n = 12, $P < 0.001$), and the $V_{0.5}$ shifted from 17.3 ± 2.9 to 6.8 ± 2.3 mV (n = 8, $P < 0.001$).

To further ensure that colocalization of the PKA to the inner membrane by AKAPs was responsible for cAMP sensitivity of the KvLQT1/IsK complex, the effects of Ht-31 peptide were investigated. Ht-31 peptide is a 24-amino acid peptide derived from a conserved amphipatic helix common to the family of AKAPs (3) that can adopt an α-helical conformation, binding to the PKA RII site and preventing PKA anchoring. The same experiments as conducted in cells coexpressing KvLQT1/IsK and mAKAP were repeated in the presence of 1 μmol/l Ht31 peptide in the intracellular pi-

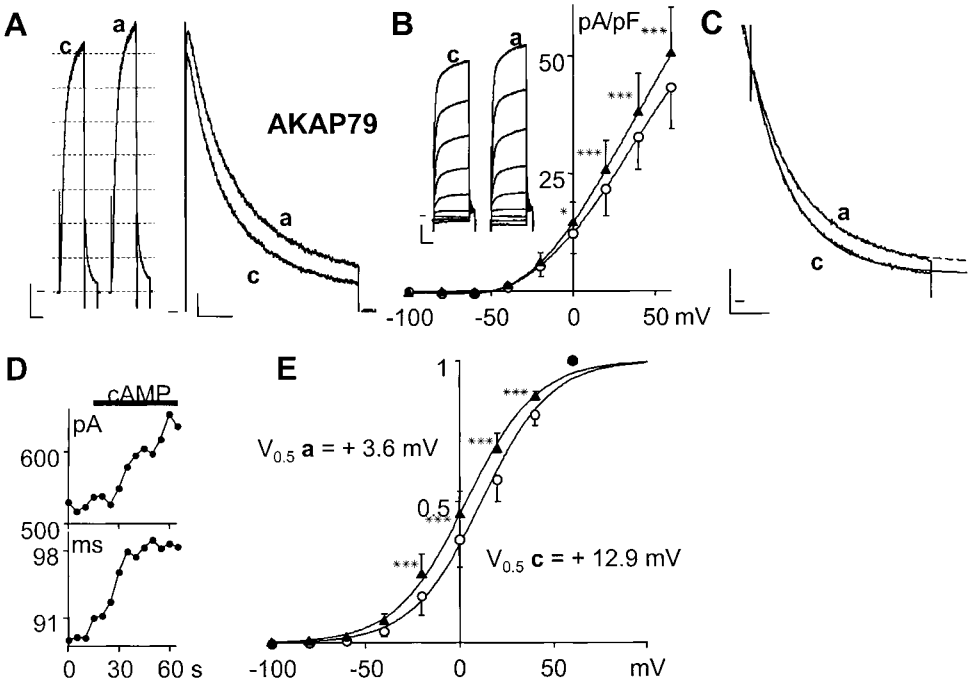

Fig. 3. Effect of AKAP79 expression on I_{Ks} sensitivity to cAMP stimulation. *A–C* are as in Fig. 1. COS-7 cells were injected with 2.5 μg/ml pCI-KvLQT1 isoform 1, 2.5 μg/ml pRC-IsK, and 3.75 μg/ml pCDNA3-A kinase anchoring protein (AKAP)79. *A*, *left*: vertical bar, 500 pA; horizontal bar, 500 ms. *Right*: vertical bar, 100 pA; horizontal bar, 100 ms. *B*: vertical bar, 500 pA; horizontal bar, 1,000 ms. *Inset*: superimposed current traces elicited by test potentials between −100 and +60 mV (increment, 20 mV; holding potential, −80 mV; vertical bar, 250 pA; horizontal bar, 1,000 ms). *C*: deactivation kinetics fitted to a single exponential (*trace c*: solid line, τ_{deact} = 105 ms; *trace a*: dashed line, τ_{deact} = 127 ms; vertical bar, 20% I_{max}; horizontal bar, 100 ms). *D*: time course of I_{Ks} tail current amplitude (*top*) and $I_{Ks}\tau_{deact}$ (*bottom*). Horizontal bar, external application of the cAMP-activating cocktail. *E*: I_{Ks} activation curves before (○) and after (▲) cAMP stimulation (n = 6). *$P < 0.05$ and ***$P < 0.001$ as assessed by two-way ANOVA.

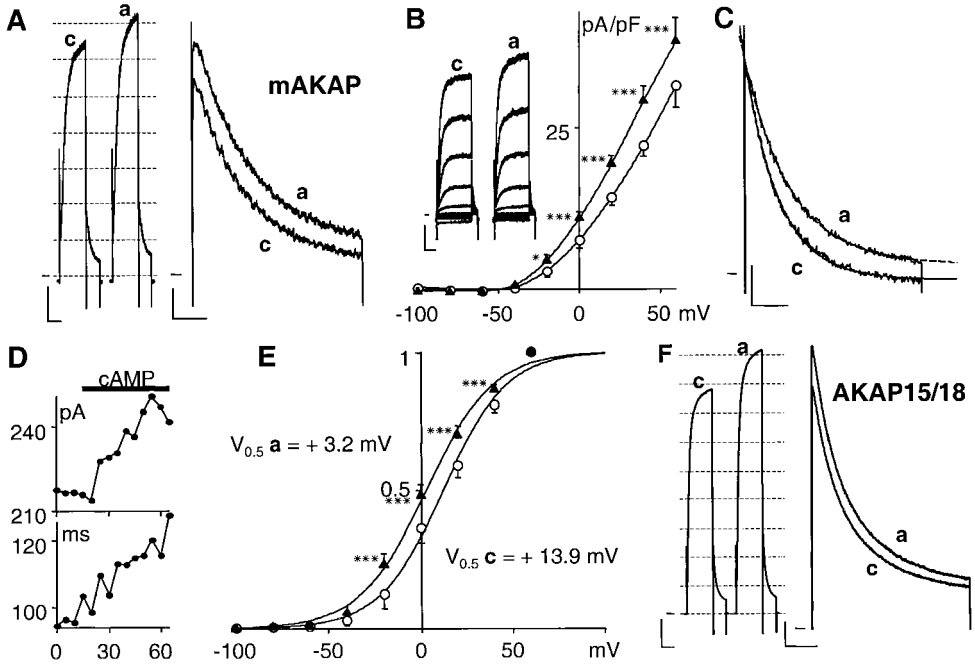

Fig. 4. Effects of a fragment of cardiac AKAP (mAKAP) or AKAP15/18 expression on I_{Ks} sensitivity to cAMP stimulation. *A–E* are as in Fig. 3. COS-7 cells were injected with 2.5 μg/ml pCI-KvLQT1 isoform 1, 2.5 μg/ml pRC-IsK, and 3.75 μg/ml pCDNA3-mAKAP. *A*, *left*: vertical bar, 200 pA; horizontal bar, 500 ms. *Right*: vertical bar, 50 pA; horizontal bar, 100 ms. *B*: vertical bar, 250 pA; horizontal bar, 1,000 ms. *Inset*: superimposed current traces elicited by test potentials between −100 and +60 mV (increment, 20 mV; holding potential, −80 mV; vertical bar, 250 pA; horizontal bar, 1,000 ms). *C*: deactivation kinetics fitted to a single exponential (*trace c*: solid line, τ_{deact} = 92 ms; *trace a*: dashed line, τ_{deact} = 117 ms; vertical bar, 20% I_{max}; horizontal bar, 100 ms). *D*: same as in Fig. 3. *E*: I_{Ks} activation curves before (○) and after (▲) cAMP stimulation (n = 6). *F*, *left*: K^+ currents recorded in a cell injected with 3.75 μg/ml pCDNA3-AKAP15/18. Same protocol as Fig. 1*A*. Vertical bar, 200 pA; horizontal bar, 500 ms. *Right*: vertical bar, 50 pA; horizontal bar, 100 ms. *$P < 0.005$ and ***$P < 0.001$ as assessed by two-way ANOVA (n = 14).

pette solution dialysing the cell. Results are summarized in Fig. 5. The basal current recorded under such conditions was not modified by Ht31 peptide. In the presence of Ht31 peptide, however, cAMP stimulation did not increase the K^+ current amplitude (tail current amplitude was 8.2 ± 1.7 pA/pF in control vs. 7.5 ± 1.5 pA/pF under cAMP, n = 7, P = NS) did not modify τ_{deact} (97.1 ± 5.9 vs. 108.5 ± 7.1 ms, n = 6, P = NS) and did not shift the activation curve ($V_{0.5}$ was 10.3. ± 5.4 vs. 7.5 ± 4.2 mV, n = 5, P = NS). Thus preventing PKA anchoring to AKAPs with Ht31 peptide suppresses its activity on KvLQT1/IsK channel complexes.

DISCUSSION

In this report, we show that AKAP expression is required for cAMP regulation of recombinant I_{Ks} in mammalian cell lines. Recombinant KvLQT1/IsK complexes expressed in mammalians cells (COS-7, HEK293, or 3T3 cells) are insensitive to cAMP stimulation in the absence of AKAP. This observation contrasts with the well-known stimulatory effects of PKA on recombinant I_{Ks} in *Xenopus* oocytes (13, 21, 26, 31). However, *Xenopus* oocytes have been shown to express an endogenous KvLQT1-like protein, which complements with exogenous IsK to form functional channel complexes (2, 22). In the absence of exogenous IsK, endogenous KvLQT1-like channel activity cannot be detected in oocytes. KvLQT1 channel proteins but not IsK possess a putative consensus phosphorylation site for PKA. Therefore, when oocytes are injected with IsK mRNA to record recombinant I_{Ks}, the target for PKA is the KvLQT1-like protein settled in its natural environment. To our knowledge, only one study (31) showed that exogenous human KvLQT1 expressed in oocytes is also sensitive to PKA phosphorylation. In that situation, however, the exogenous KvLQT1 profits in the same environment as the endogenous KvLQT1-like protein. Our study, which was performed in an expression system containing no endogenous KvLQT1 expression, demonstrates that exogenous KvLQT1 is not sensitive to PKA. In this connection, it was previously shown that the sensitivity to regulation by PKA of Kv1.3 channels is partially lost when recombinant channels are expressed in mammalian HEK293 host cells (15). Similarly, Zong et al. (34) reported that the L-type Ca^{2+} channel reconstituted in HEK293 cells does not display the same extent of sensitivity to modulation by cAMP-dependent phosphorylation as the native channel in myocytes. Likewise, Gerlach et al. (8) found that hIK1 K^+ channels are activated by PKA when endogenously expressed and that this regulation is recapitulated in *Xenopus* oocytes but not in HEK293 cells. Interestingly, cAMP regulation of *KCNQ2* and *KCNQ3* gene products, which are highly homologous to KvLQT1, does not require coexpression of an AKAP in mammalian cells (24).

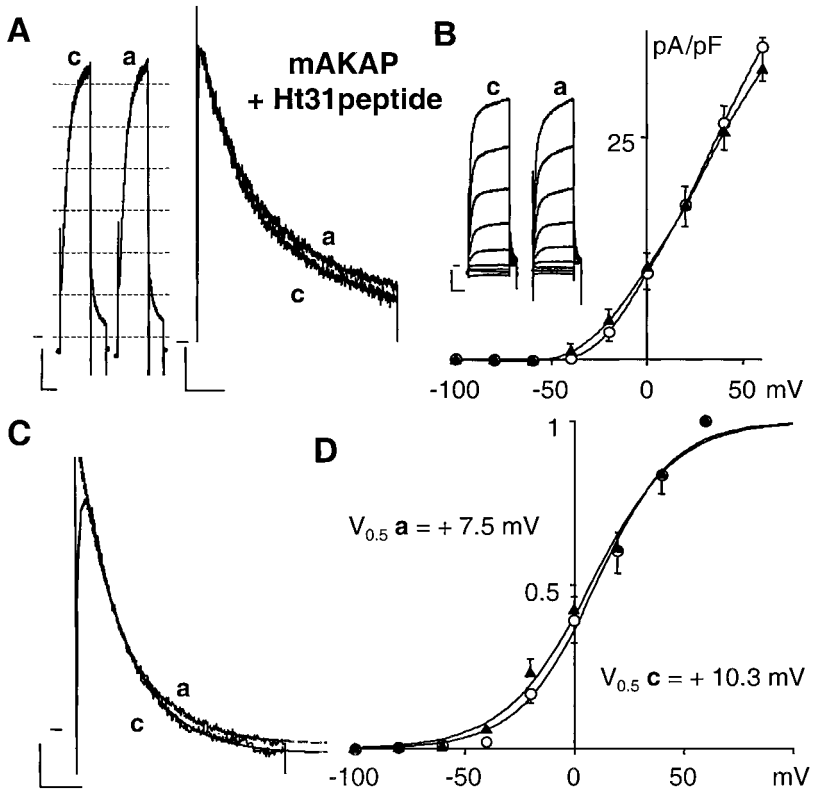

Fig. 5. Ht31 peptide prevents cAMP enhancement of I_{Ks} in cells expressing mAKAP. *A–D* are as in Fig. 1. COS-7 cells were injected with 2.5 μg/ml pCI-KvLQT1 isoform 1, 2.5 μg/ml pRC-IsK, and 3.75 μg/ml pCDNA3-mAKAP. Cells were dialyzed with 1 μmol/l Ht31 peptide diluted in the pipette solution. *A*, *left*: vertical bar, 200 pA; horizontal bar, 500 ms. *Right*: vertical bar, 50 pA; horizontal bar, 100 ms. *B*: *I-V* relation for I_{Ks} in control conditions (○) and after cAMP (▲, $n = 11$). *Inset*: vertical bar, 500 pA; horizontal bar, 1,000 ms. *C*: deactivation kinetics were fitted to a single exponential (*trace c*: solid line, $\tau_{deact} = 97$ ms; *trace a*: dashed line, $\tau_{deact} = 104$ ms; vertical bar, 20% I_{max}; horizontal bar, 100 ms). *D*: I_{Ks} activation curves before (○) and after (▲) cAMP stimulation ($n = 6$).

In mammalian cells transfected with human KvLQT1/IsK complexes, coexpression of AKAPs and biologically active fragments restored regulation of recombinant I_{Ks} by PKA. Functional analysis of AKAPs began when Rosenmund et al. (19) applied electrophysiological techniques in concert with the perfusion of Ht-31 peptide to assess the role of AKAPs in cultured hippocampal neurons. Since 1994, studies have shown that AKAPs are involved in ionic channel regulation in a variety of tissues (6). Cardiac AKAP15/18 has been shown to be required for PKA-dependent potentiation of L-type calcium channels (9, 11). In mammalian cells expressing AKAP79, mAKAP, or AKAP15/18, PKA increased the amplitude of recombinant I_{Ks}, shifted its activation curve, and slowed its deactivation kinetics. These effects recapitulate those produced by cAMP stimulation on endogenous I_{Ks} in cardiac myocytes (28, 32). However, in cardiac myocytes, cAMP stimulation with isoproterenol almost doubles the I_{Ks} tail amplitude (23, 28, 29, 32). In *Xenopus* oocytes expressing exogenous KvLQT1, the addition of 10 μmol/l forskolin plus 100 μmol/l IBMX increases the I_{Ks} peak current by 44% (31). In our study, 10 μmol/l forskolin plus 400 μmol/l cpt-cAMP increased the recombinant I_{Ks} tail amplitude by only 15% in COS-7 cells expressing AKAP79, by 16% in cells expressing mAKAP, and by 22% in cells expressing AKAP15/18. Although these effects were fully prevented by Ht31 peptide and although they were accompanied by significant modifications in the activation curve and deactivation kinetics, they remained modest in amplitude relatively to what is usually observed in myocytes. We interpret this difference as an indication that host mammalian cells are still missing an additional partner in the channel complex. Further investigation should be directed to identify this putative partner.

The main function of AKAPs is to contribute to PKA compartimentalization in the vinicity of PKA targets. KvLQT1 channel proteins are mainly expressed in cardiac and epithelial tissues but are not abundant in the central nervous system (5). Despite this tissue distribution, cAMP regulation of recombinant I_{Ks} was restored when either the neuronal AKAP79 or the cardiac mAKAP or AKAP15/18 were coexpressed, suggesting a low specificity for AKAPs to permit PKA regulation of KvLQT1. Critical RII-binding regions in several AKAPs have been identified and consist in each case of a single amphipathic helix (18). Interestingly, the AKAP binding of PKA in the heart may be regulated by RII autophophorylation (33). A short peptide containing the RII-binding amphipathic helix of the AKAP Ht31 has been shown to bind RII with nanomolar affinity and has been termed an "anchoring inhibitor peptide" on the basis of its ability to competitively inhibit RII-AKAP interactions. Microinjection of Ht31 peptide displaces the kinase from the anchoring sites and attenuates ion flow through skeletal muscle L-type Ca^{2+} channels (11) and α-amino-3-hydroxy-5-methylisotazole propione acid-kainate glutamate receptor ion channels (19).

Given the importance of I_{Ks} in cardiac action potential repolarization and its role in long Q-T syndrome, understanding the regulation of human I_{Ks} may shed light on its physiological and pathophysiological roles.

The ability to up- or downregulate I_{Ks} is a means of controlling the duration of action potential in the human heart. I_{Ks} is generated by a complex protein assembly composed of KvLQT1 isoform 1 (the channel pore), KvLQT1 isoform 2 (a dominant negative protein), and IsK (the channel regulator). The present data provide support for the formation of signaling complexes in which protein kinases that regulate phosphorylation status of KvLQT1 isoform 1 are maintained in proximity to their target protein. Our results therefore suggest that, in the human heart, I_{Ks} is generated by a protein assembly composed of KvLQT1 isoform1, KvLQT1 isoform2, IsK, and a anchoring protein that permits its β-adrenergic regulation.

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