PKA-independent activation of $I_f$ by cAMP in mouse sinoatrial myocytes

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Abstract

Hyperpolarization-activated, cyclic nucleotide-sensitive (HCN4) channels produce the “funny current,” $I_f$, which contributes to spontaneous pacemaking in sinoatrial myocytes (SAMs). The C-terminus of HCN channels inhibits voltage-dependent gating, and cAMP binding relieves this “autoinhibition.” We previously showed 1) that autoinhibition in HCN4 can be relieved in the absence of cAMP in some cellular contexts and 2) that PKA is required for β adrenergic receptor (βAR) signaling to HCN4 in SAMs. Together, these results raise the possibility that native HCN channels in SAMs may be insensitive to direct activation by cAMP. Here, we examined PKA-independent activation of $I_f$ by cAMP in SAMs. We observed similar robust activation of $I_f$ by exogenous cAMP and Rp-cAMP (an analog that cannot activate PKA). Thus PKA-dependent βAR-to-HCN signaling does not result from cAMP insensitivity of sinoatrial HCN channels and might instead arise via PKA-dependent limitation of cAMP production and/or cAMP access to HCN channels in SAMs.

Introduction

Hyperpolarization-activated, cyclic nucleotide-sensitive (HCN4) channels produce the cardiac “funny current,” $I_f$, which contributes to spontaneous pacemaker activity in sinoatrial myocytes (SAMs). HCN channels have a conserved cyclic nucleotide-binding domain (CNBD) in the C-terminus which inhibits voltage-dependent gating. cAMP binding to the CNBD relieves this “autoinhibition,” causing a depolarizing shift in the voltage dependence of activation. We recently observed that autoinhibition of HCN4 (the predominant sinoatrial HCN isoform) can be relieved in the absence of ligand in some cellular contexts, rendering the channels insensitive to cAMP.

β adrenergic receptor (βAR) stimulation potentiates $I_f$ via a depolarizing shift in the voltage dependence of activation. It is generally assumed that direct cAMP binding to HCN4 mediates this βAR activation of $I_f$. However, we previously showed that βAR signaling to HCN channels in SAMs requires PKA activity, and that PKA phosphorylation of heterologously-expressed HCN4 channels causes a depolarizing shift in voltage dependence, which is similar in magnitude to the shifts produced by βAR stimulation or cAMP binding. These results suggest a model in which βAR-generated cAMP activates $I_f$ via PKA-dependent phosphorylation of the native sinoatrial HCN channels. However, indirect, mechanisms for PKA-dependent regulation of $I_f$ are also possible, and the mechanistic basis for the PKA requirement in βAR-to-HCN signaling in SAMs is not known.

Taken together our findings of tunable cAMP sensitivity of HCN4 and of PKA-dependence in βAR-to-HCN signaling raise the possibility that native HCN channels in mouse SAMs may be insensitive to direct activation by cAMP. In this short follow-up study, we evaluated the ability of cAMP to activate $I_f$ in mouse SAMs in the absence of PKA activity.

Keywords: sinoatrial node, If, hyperpolarization-activated cyclic nucleotide-sensitive channel, HCN4, cyclic nucleotide-binding domain, protein kinase A

Abbreviations: βAR, β adrenergic receptor; CHO, Chinese hamster ovary cell; CNBD, cyclic nucleotide-binding domain; HCN, hyperpolarization-activated, cyclic nucleotide-sensitive channel; HEK, human embryonic kidney cell; ISO, isoproterenol; PDE, phosphodiesterase; Rp-cAMPS, Rp-adenosine cyclic 3’,5’-phosphorothioate; SAM, sinoatrial myocyte

Submitted: 06/03/13
Accepted: 06/05/13
http://dx.doi.org/10.4161/chan.25293

Addendum to: Liao Z, Lockhead D, St Clair JR, Larson ED, Wilson CE, Proenza C. Cellular context and multiple channel domains determine cAMP sensitivity of HCN4 channels: ligand-independent relief of autoinhibition in HCN4. J Gen Physiol 2012; 140:557-66; PMID:23109717; http://dx.doi.org/10.1085/jgp.201210858

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ARTICLE ADDENDUM

Results and Discussion

$I_f$ was recorded from acutely dissociated mouse SAMs in whole cell voltage clamp recordings. Cells were held at −50 mV, and $I_f$ was elicited by 3-sec test pulses from −60 to −170 mV in 10 mV increments (Fig. 1A). To determine whether cAMP can activate native HCNs in SAMs independent of PKA activity, we compared the effects on the midpoint activation voltage ($V_{1/2}$) of $I_f$ in response to intracellular dialysis with cAMP or Rp-adenosine cyclic 3’5’-phosphorothioate (Rp-cAMPS), a cAMP analog that cannot activate PKA but can activate $I_f$ in excised inside-out membrane patches from rabbit SAMs. We found that cAMP and Rp-cAMPS produced nearly identical depolarizing shifts in the midpoint activation voltage ($V_{1/2}$) of $I_f$ in mouse SAMs when applied at either

![Figure 1. Similar effects of cAMP and Rp-cAMPS on $I_f$ in sinoatrial myocytes. (A) Representative $I_f$ whole cell current families recorded from SAMs in control (Tyrodes), 1 mM cAMP, or 1 mM Rp-cAMPS. Red traces indicate currents at −100 mV to illustrate similar shift in voltage dependence in the presence of cAMP or Rp-cAMPS. Scale bars, 250 ms 200 pA for control and 1 mM cAMP, 250 ms, and 100 pA for 1 mM Rp-cAMPS (B) Average normalized conductance-voltage plots for $I_f$ in Tyrodes (black circles), 1 mM cAMP (red circles), or 1 mM Rp-cAMPS (red triangles). (C) Average normalized conductance-voltage plots for $I_f$ in Tyrodes (black circles), 100 μM cAMP (red circles), or 100 μM Rp-cAMPS (red triangles). (D) Average midpoint activation voltages for $I_f$ in Tyrodes, 1 μM ISO, or the indicated concentrations of cAMP or Rp-cAMPS. Asterisks indicate p < 0.05 vs. Tyrodes, ns indicates p > 0.05.](image-url)
Materials and Methods

Animal procedures were performed in accordance with protocols approved by the IACUC at the University of Colorado Denver, Anschutz Medical Campus. SAMs were isolated from adult male C57BL/6J mice as previously described.\textsuperscript{2,3}

Whole-cell voltage-clamp recordings of $I_v$, from SAMs conducted as previously reported.\textsuperscript{3,7} Cells were perfused (1-2 ml/min) with Tyrode’s solution (in mM, 140 NaCl, 5.4 KCl, 1.2 KH$_2$PO$_4$, 5 HEPES, 5.55 glucose, 1.8 CaCl$_2$; pH adjusted to 7.4 with NaOH) containing 1 mM BaCl$_2$. Recording pipettes had resistances of -1.5-3.0 MΩ when filled with an intracellular solution consisting of (in mM) 135 potassium aspartate, 6.6 sodium phosphocreatine, 1 MgCl$_2$, 1 CaCl$_2$, 10 HEPES, 10 EGTA, 4 Mg-ATP; pH adjusted to 7.2 with KOH. cAMP (Sigma-Aldrich A6885) or Rp-adenosine-3’,5’-cyclic monophosphorothioate sodium salt (Rp-cAMPS; BioLog A 002 S) were added to the intracellular solution at the indicated concentrations. Reported voltages were corrected for a calculated -14 mV junction potential error. Conductance was calculated from inward currents using the equation $G = I/(V - V_r)$, where $G$ is conductance, $I$ is the time-dependent inward current at a given voltage, $V$ and $V_r$ is the reversal potential for $I_v$ (−30 mV\textsuperscript{2,3,7}). Conductances were subsequently plotted as a function of voltage and fit with a Boltzmann equation to determine midpoint activation voltages ($V_{1/2}$).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by NIH grant HL088427 (to CP). EDL was partially supported by NINDS T32NS007083.

Table 1. Midpoint activation voltages for $I_v$ in mouse sinoatrial myocytes

| Tyroses | $V_{1/2}$ control (mV) | n |
|---------|------------------------|---|
| ISO     | −112 ± 1.9*            | 7 |
| 3 mM cAMP | −114 ± 1.9*          | 7 |
| 1 mM cAMP | −112 ± 1.9*        | 10|
| 1 mM Rp-cAMP | −112 ± 1.9*      | 10|
| 100 μM cAMP | −121 ± 2.6*    | 14|
| 100 μM Rp-cAMP | −124 ± 2.2*   | 14|

*p < 0.05 vs. control, *p < 0.05 in comparison with each other. One-way ANOVAs with Student-Newman-Keuls post-test.

References

1. Wangier BJ, DeGennaro M, Santoro B, Siegelbaum SA, Tibbs GR. Molecular mechanism of cAMP modulation of HCN pacemaker channels. Nature 2001; 411:805-10; PMID:11499600; http://dx.doi.org/10.1038/35081088

2. Liao Z, Lockhead D, St Clair JR, Larson ED, Wilson CE, Proenza C. Cellular context and multiple channel domains determine cAMP sensitivity of HCN4 channels: ligand-independent relief of auto-inhibition in HCN4. J Gen Physiol 2012; 140:557-66; PMID:23109717; http://dx.doi.org/10.1085/jgp.201210858

3. Liao Z, Lockhead D, Larson ED, Proenza C. Phosphorylation and modulation of hyperpolarization-activated HCN4 channels by protein kinase A in the mouse sinoatrial node. J Gen Physiol 2010; 136:247-58; PMID:20713547; http://dx.doi.org/10.1085/jgp.201010488

4. Rothermel JD, Parker Botello LH. A mechanistic and kinetic analysis of the interactions of the diasteroisomers of adenosine 3’,5’-(cyclic)phosphorothioate with purified cyclic AMP-dependent protein kinase. Biochem J 1988; 251:757-62; PMID:2831646

5. Bois P, Renaudon B, Baruscotti M, Lenfant J, DiFrancoce D. Activation of f-channels by cAMP analogues in macropatches from rabbit sino-atrial node myocytes. J Physiol 1997; 501:565-71; PMID:9218217; http://dx.doi.org/10.1111/j.1469-7793.1997.565bm.x

6. Willoughby D, Cooper DM. Organization and Ca²⁺-regulation of adenyl cyclases in cAMP microdomains. Physiol Rev 2007; 87:965-1010; PMID:17615394; http://dx.doi.org/10.1152/physrev.00049.2006

7. Matrick P, Parrington J, Oda E, Simpson A, Collins T, Terrar D. Ca²⁺-stimulated adenyl cyclase isoform AC1 is preferentially expressed in guinea-pig sino-atrial node cells and modulates the If pacemaker current. J Physiol 2007; 582:1195-203; PMID:17540702; http://dx.doi.org/10.1111/j.1469-8137.2007.33439

8. Omori K, Koterz J. Overview of PDEs and their regulation. Circ Res 2007; 100:309-27; PMID:17367970; http://dx.doi.org/10.1161/01.RES.0000256354.95791.f1

9. Oliveira RF, Terrin A, Di Benedetto G, Cannon RC, Koh W, Kim M, et al. The role of type 4 phosphodiesterases in generating microdomains of cAMP; large scale stochastic simulations. PLoS One 2010; 5:e11725; PMID:20661441; http://dx.doi.org/10.1371/journal.pone.0011725

10. Terrin A, Monterisi S, Strangerlin A, Zoccarato A, Koschinski A, Nardo NC, et al. PKA and PDE4D3 anchoring to AKAP9 provides distinct regulation of cAMP signals at the centrosome. J Cell Biol 2012; 198:607-21; PMID:22998311; http://dx.doi.org/10.1083/jcb.201201059

11. Barnes AP, Livera G, Huang P, Sun C, O’Neal WK, Conti M, et al. Phosphodiesterase 4D forms a cAMP phosphodiesterase in generating microdomains of cAMP; large scale stochastic simulations. PLoS One 2010; 5:e11725; PMID:20661441; http://dx.doi.org/10.1371/journal.pone.0011725

12. Jurevicus J, Skeberdis VA, Fischmeister R. Role of cyclic nucleotide phosphodiesterase isoforms in cAMP compartmentation following beta2-adrenergic stimulation of ICA, I in frog ventricular myocytes. J Physiol 2003; 551:239-52; PMID:12883180; http://dx.doi.org/10.1113/jphysiol.2003.045211

13. Hua R, Adamczyk A, Robbins C, Ray G, Rose RA. Distinct patterns of constitutive phosphodiesterase activity in mouse sinoatrial node and atrial myocardium. PLoS One 2012; 7:e47652; PMID:23077656; http://dx.doi.org/10.1371/journal.pone.0047652

14. Galindo-Tovar A, Kaumann AJ. Phosphodiesterase-4 blunts inotropism and arrhythmias but not sinoatrial tachycardia of (−)-adrenaline mediated through mouse cardiac beta(1)-adrenoceptors. Br J Pharmacol 2008; 153:710-20; PMID:18084359; http://dx.doi.org/10.1038/sj.bjp.0707631

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15. Kaumann AJ, Galindo-Tovar A, Escudero E, Vargas ML. Phosphodiesterases do not limit beta1-adrenergic receptor-mediated sinoatrial tachycardia: evidence with PDE3 and PDE4 in rabbits and PDE1-5 in rats. Naunyn Schmiedeberg’s Arch Pharmacol 2009; 379:421-30; PMID:19693491; http://dx.doi.org/10.1007/s00210-009-0445-5

16. Vinogradova TM, Sirenko S, Lyashkov AE, Younes A, Li Y, Zhu W, et al. Constitutive phosphodiesterase activity restricts spontaneous beating rate of cardiac pacemaker cells by suppressing local Ca2+ releases. Circ Res 2008; 102:761-9; PMID:18276917; http://dx.doi.org/10.1161/CIRCRESAHA.107.161679

17. Liao Z, St Clair JR, Larson ED, Proenza C. Myristoylated peptides potentiate the funny current (I(f)) in sinoatrial myocytes. Channels (Austin) 2011; 5:115-9; PMID:21150293; http://dx.doi.org/10.4161/chan.5.2.14195

18. Mangoni ME, Nargeot J. Properties of the hyperpolarization-activated current (I(h)) in isolated mouse sino-atrial cells. Cardiovasc Res 2001; 52:51-64; PMID:11557233; http://dx.doi.org/10.1016/S0008-6363(01)00370-4