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

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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 than 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.

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

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Introduction

Hyperpolarization-activated, cyclic nucleotide-sensitive (HCN) 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.[1] 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.[2]

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.[3] 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.

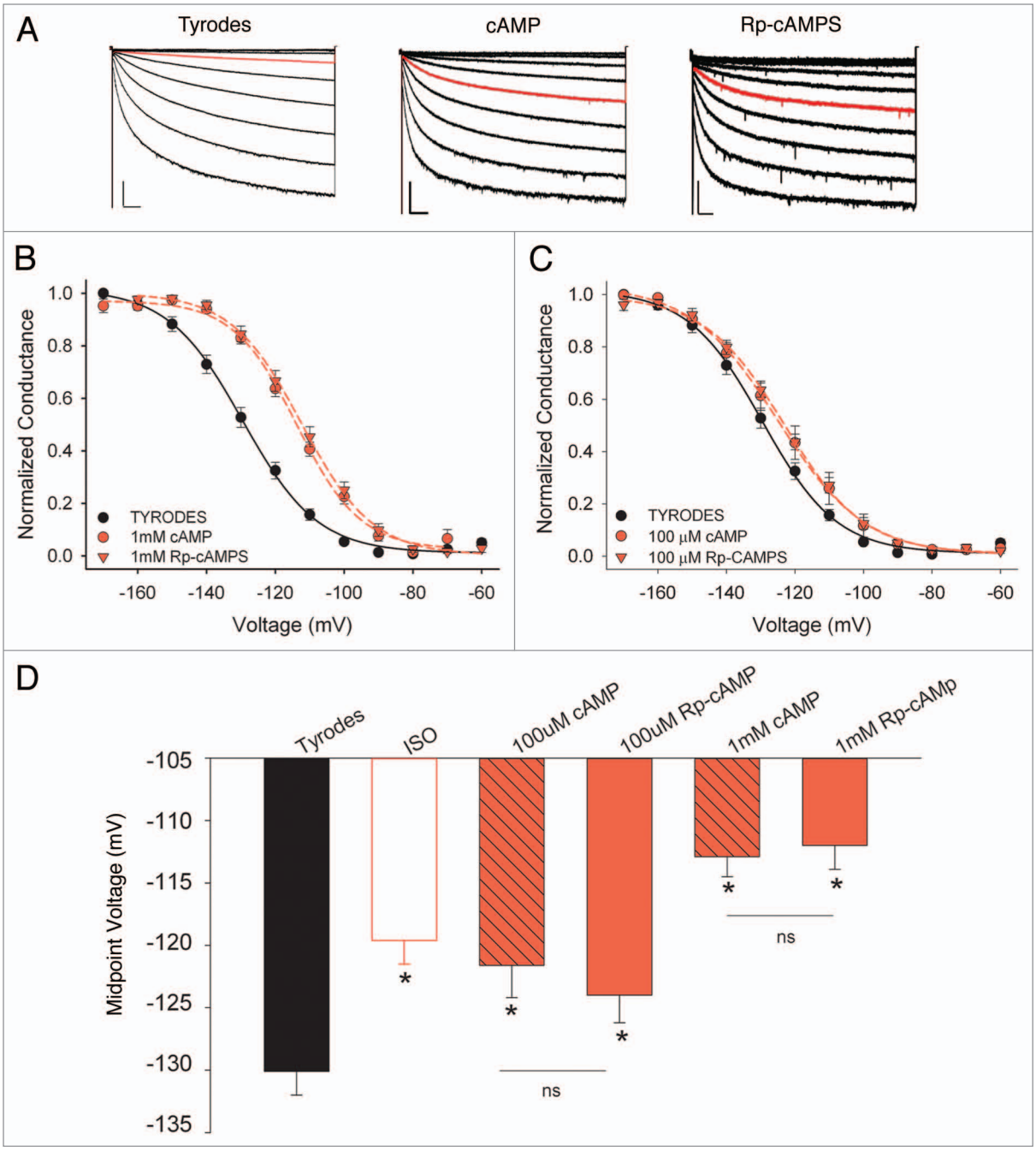

Figure 1. Similar effects of cAMP and Rp-cAMPS on I_f in sinoatrial myocytes. (**A**) Representative I_f whole cell current families recorded from SAMs in control (Tyrodes), 1 mM cAMP, or 1 mM Rp-cAMPS. Red traces indicate currents at –100 mV to illustrate similar shift in voltage dependence in the presence 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-cAMP (**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 $p > 0.05$.

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[4] but can activate I_f in excised inside-out membrane patches from rabbit SAMs.[5] 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

Table 1. Midpoint activation voltages for I_f in mouse sinoatrial myocytes

	$V_{1/2}$ control (mV)	n
Tyrodes	–130.1 ± 1.9	17
ISO	–119.6 ± 1.9*	17
3 mM cAMP	–114.1 ± 1.9*,†	7
1 mM cAMP	–112.9 ± 1.6*,†	10
1 mM Rp-cAMP	–112.0 ± 1.9*,†	10
100 uM cAMP	–121.6 ± 2.6*	14
100 uM Rp-cAMP	–124.0 ± 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.

a saturating (1 mM) or sub-saturating (100 μM) concentration (**Fig. 1B and C**; **Table 1**). The $V_{1/2}$ values for 1 mM cAMP or Rp-cAMPS were significantly more depolarized than the $V_{1/2}$ produced by the βAR agonist, isoproterenol (ISO; 1 μM), whereas $V_{1/2}$ values in 100 μM Rp-cAMPS or cAMP were statistically similar to those produced by ISO (**Table 1**).

These data demonstrate that PKA activity is not required for cAMP stimulation of native HCN channels in mouse sinoatrial myocytes under the same recording conditions in which we observed that PKA activity is required for βAR-mediated activation of I_f. Thus, the requirement for PKA in βAR-to-HCN signaling in SAMs does not result from cAMP insensitivity of sinoatrial HCN channels. Rather, it most likely arises via limitation of cAMP production and/or restriction of cAMP access to HCN channels. A simple model for PKA-dependent cAMP production is difficult to conceive given that the known PKA-sensitive adenylate cyclase (AC) isoforms are inhibited rather than activated by PKA[6] (although a more complicated scenario is suggested by the observation that Ca^{2+}-activated ACs have been shown to regulate I_f in guinea pig SAMs[7]). On the other hand, PKA activates the cardiac phosphodiesterases, PDE3 and PDE4[8] and PDEs have been shown to form restricted cAMP signaling domains in a variety of cellular systems[9-12] and to regulate both I_f[13] and sinoatrial pacemaking rate.[14-16] Quantitative description of the roles of ACs and PDEs in βAR regulation of I_f in SAMs awaits future studies.

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.[3,17]

Whole-cell voltage-clamp recordings of I_f from SAMs were conducted as previously reported.[3,17] Cells were perfused (1–2 ml/min) with Tyrode's solution (in mM, 140 NaCl, 5.4 KCl, 1.2 KH_2PO_4, 5 HEPES, 5.55 glucose, 1 $MgCl_2$, 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_f (–30 mV[3,18]). 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.

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