



Modeling the chronotropic effect of isoprenaline on rabbit sinoatrial node

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Introduction: β -adrenergic stimulation increases the heart rate by accelerating the electrical activity of the pacemaker of the heart, the sinoatrial node (SAN). Ionic mechanisms underlying the actions of β -adrenergic stimulation are not yet fully understood. Isoprenaline (ISO), a β -adrenoceptor agonist, shifts voltage-dependent I_f activation to more positive potentials resulting in an increase of I_f , which has been suggested to be the main mechanism underlying the effect of β -adrenergic stimulation. However, ISO has been found to increase the firing rate of rabbit SAN cells when I_f is blocked. ISO also increases I_{CaL} , I_{st} , I_{Kr} , and I_{Ks} ; and shifts the activation of I_{Kr} to more negative potentials and increases the rate of its deactivation. ISO has also been reported to increase the intracellular Ca^{2+} transient, which can contribute to chronotropy by modulating the “ Ca^{2+} clock.” The aim of this study was to analyze the ionic mechanisms underlying the positive chronotropy of β -adrenergic stimulation using two distinct and well established computational models of the electrical activity of rabbit SAN cells. **Methods and results:** We modified the Boyett et al. (2001) and Kurata et al. (2008) models of electrical activity for the central and peripheral rabbit SAN cells by incorporating equations for the known dose-dependent actions of ISO on various ionic channel currents (I_{CaL} , I_{st} , I_{Kr} , and I_{Ks}), kinetics of I_{Kr} and I_f , and the intracellular Ca^{2+} transient. These equations were constructed from experimental data. To investigate the ionic basis of the effects of ISO, we simulated the chronotropic effect of a range of ISO concentrations when ISO exerted all its actions or just a subset of them. **Conclusion:** In both the Boyett et al. and Kurata et al. SAN models, the chronotropic effect of ISO was found to result from an integrated action of ISO on I_{CaL} , I_f , I_{st} , I_{Kr} , and I_{Ks} , among which an increase in the rate of deactivation of I_{Kr} plays a prominent role, though the effect of ISO on I_f and $[Ca^{2+}]_i$ also plays a role.

Keywords: sinoatrial node, isoprenaline, action potential

INTRODUCTION

β -Adrenergic stimulation increases the heart rate through accelerating the spontaneous activity of the pacemaker of the heart, the sinoatrial node (SAN; Abramochkin et al., 2009). It is believed that this occurs through β -adrenoceptor mediated modulation of ionic currents that contribute to pacemaker activity; however the precise ionic mechanisms underlying the effect of β -adrenergic stimulation are not yet fully elucidated. Experiments have shown that isoprenaline (ISO), a β -adrenergic agonist, increases the L-type calcium current (I_{CaL} ; Noma et al., 1980; Walsh et al., 1988; Zaza et al., 1996; Vinogradova et al., 2002; Ke et al., 2007; Alig et al., 2009), delayed rectifier potassium current (both I_{Kr} and I_{Ks} ; Walsh et al., 1988; Duchatelle-Gourdon et al., 1989; Giles et al., 1989; Yazawa and Kameyama, 1990; Freeman and Kass, 1993; Lei et al., 2000, 2002; Ke et al., 2007; Vinogradova et al., 2008), and shifts voltage-dependent activation of the hyperpolarization activated current (I_f) toward positive potentials resulting in an increase of

I_f (Brown et al., 1979; Cai et al., 1995; DiFrancesco, 1995; Zaza et al., 1996; Accili et al., 1997a,b; Bucchi et al., 2003; Barbuti et al., 2007; Alig et al., 2009; Baruscotti et al., 2010; Liao et al., 2010). As I_f has been regarded to be a major pacemaker current in mammalian pacemaker cells, an increase in I_f has been suggested to be the main mechanism underlying the positive chronotropic effect of β -adrenergic stimulation (DiFrancesco, 1995, 2010; Zaza et al., 1996; DiFrancesco and Borer, 2007; Liao et al., 2010). However, ISO has been found to increase the firing rate of the rabbit SAN when I_f was blocked by Cs^+ (Cai et al., 1995), suggesting that I_f enhancement may not be predominantly responsible for the positive chronotropic action of ISO. Previous studies have shown that ISO increases the amplitude of the systolic rise of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiac cells (Ju and Allen, 1999; Huser et al., 2000; Shannon et al., 2004; Maltsev and Lakatta, 2009; Wu et al., 2009). This raises the possibility that changes to $[Ca^{2+}]_i$ with ISO might contribute to the increase in firing rate via the

“Ca²⁺ clock” mechanism (Vinogradova et al., 2002, 2008; Maltsev and Lakatta, 2009). A number of studies have shown that interventions altering [Ca²⁺]_i change the firing rate of pacemaker cells (Hagiwara, 1989; Ju and Allen, 1999; Huser et al., 2000; Shannon et al., 2004; Vinogradova et al., 2008; Maltsev and Lakatta, 2009; Wu et al., 2009). In both mammalian cardiac cells (Hagiwara, 1989; Huser et al., 2000; Vinogradova et al., 2002, 2008; Maltsev and Lakatta, 2009; Wu et al., 2009) and amphibian pacemaker cells in which *I_f* is absent (Ju and Allen, 1999), it has been found that the firing rate was dependent on the amplitude of the [Ca²⁺]_i transient: agents modifying SR Ca²⁺ release consequently affect the firing rate. β-Adrenergic stimulation increases the amplitude of the [Ca²⁺]_i transient. It has been argued that much of the increase in firing rate caused by β-stimulation seems to occur through the “Ca²⁺ clock” mechanism (Vinogradova et al., 2002, 2008; Maltsev and Lakatta, 2009). ISO has also been reported to increase the amplitude of the inward sustained current (*I_{st}*; Guo et al., 1995, 1997; Shinagawa and Noma, 2000; Toyoda et al., 2005), and the amplitude of the *I_{Kr}* and *I_{Ks}* (Lei et al., 2000, 2002). It has also been found to shift the activation curve of *I_{Kr}* toward more negative membrane potentials and to increase its rate of deactivation (Yazawa and Kameyama, 1990; Lei et al., 2000, 2002; Ke et al., 2007). As *I_{st}* is activated over a membrane potential range between −70 and −50 mV (Guo et al., 1995, 1997; Shinagawa and Noma, 2000), increases in *I_{st}* might play a role in accelerating the rate of pacemaker activity. An increase in the rate of deactivation of *I_{Kr}* decreases the outward current, resulting in a relatively larger net inward current during diastolic depolarization, which could also contribute to the increase of the rate of spontaneous activity. In this study, we modified existing models of the electrical activity of central and peripheral rabbit SAN cells (Boyett et al., 2001; Kurata et al., 2008) in order to simulate the positive chronotropic effect of ISO and study the underlying ionic basis for the actions of ISO using a computational approach. We have incorporated equations for concentration-dependent actions of ISO on the macroscopic conductance of *I_{CaL}*, *I_{Kr}* and *I_{Ks}*, *I_{st}* and to describe changes in the kinetics of *I_f* and *I_{Kr}*, and in [Ca²⁺]_i. These equations have been constructed from experimental data. Using the models, the simulated effect of ISO shows concentration-dependence, which is quantitatively consistent with experimental recordings. To investigate the underlying ionic basis the effect of ISO, we examined its effects over a range of concentrations on the currents responsible for the pacemaker activity of the SAN, and we have investigated the individual role of actions of ISO on currents of *I_{CaL}*, *I_f*, *I_{Kr}*, *I_{Ks}*, *I_{st}*, and [Ca²⁺]_i. We found that the chronotropic effect of ISO reflects integrated actions on ionic currents *I_{CaL}*, *I_f*, *I_{Kr}*, *I_{Ks}*, *I_{st}*, and [Ca²⁺]_i, amongst which the increase in the rate of deactivation of *I_{Kr}* appears to be particularly important, whilst the effect of ISO on *I_f* and [Ca²⁺]_i also plays a role.

MODEL DEVELOPMENT

Based on the voltage clamp experimental data on the kinetics of ionic channels and regional differences in the ionic current densities, well established action potentials models of the center and periphery of the rabbit SAN cells have been developed (Zhang et al., 2000; Boyett et al., 2001; Kurata et al., 2008). The Zhang et al. (2000) models were upgraded by incorporating an inward

sustained current (*I_{st}*) and intracellular Ca²⁺ regulation equations (Boyett et al., 2001). In this study, we modified the models of Boyett et al. (2001) and Kurata et al. (2008) by including equations describing the actions of ISO on *I_{CaL}*, *I_f*, *I_{Kr}*, *I_{Ks}*, *I_{st}*, and [Ca²⁺]_i.

INCREASE OF *I_{CaL}*

Isoprenaline was found, experimentally, to increase *I_{CaL}* without a significant change in its kinetics (Zaza et al., 1996). The increase in *I_{CaL}* is dose dependent. The equation describing the dose-dependent increase of *I_{CaL}* was constructed from experimental data of Zaza et al. (1996) obtained from rabbit SAN cells, which take the form:

$$f_{Ca} = f_{Ca,max} \frac{[ISO]}{K_{0.5,Ca} + [ISO]} \quad (1)$$

Where *f_{Ca}* is the percentage of increase of *I_{CaL}*. By fitting Eq. 1 to the data of Zaza et al. (1996; circles) and Vinogradova et al. (2002; triangles) as shown in **Figure 1A**, we obtained the best fit values for *f_{Ca,max}* (the maximum percentage increase *I_{CaL}*) and *K_{0.5,Ca}* (the ISO concentration required to half-maximally increase of *I_{CaL}*) as 0.54 (i.e., 54%) and 7 nM, respectively. The obtained *K_{0.5,Ca}* (7 nM) matches to the experimental data of EC₅₀ of ISO on *I_{CaL}* (Zaza et al., 1996). The solid line in **Figure 1A** shows the relationship between the increase in *I_{CaL}* and the ISO concentration predicted by Eq. 1.

SHIFT OF THE ACTIVATION CURVE OF *I_f*

Isoprenaline increases *I_f* by shifting its voltage-dependent activation curve toward more positive membrane potentials (Zaza et al., 1996; Accili et al., 1997a,b). The equation to describe the shift, *S_f* (in mV), was constructed from the experimental data of Zaza et al. (1996) obtained from rabbit SAN cells as shown below:

$$S_f = S_{f,max} \frac{[ISO]^{n_f}}{K_{0.5,f}^{n_f} + [ISO]^{n_f}} \quad (2)$$

By fitting the Eq. 2 to the data of Zaza et al. (1996; **Figure 1B**, circles), we obtained the best fit values for *S_{f,max}* (the maximum shift), *K_{0.5,f}* (the ISO concentration required to half-maximally shift the activation curve), and *n_f* (the Hill coefficient) as 9.62 mV, 13.5 nM, and 0.392, respectively, which are close to those obtained by Zaza et al. (1996). The solid line in **Figure 1B** shows the relationship between the shift of *I_f* activation curve and the ISO concentration predicted by Eq. 2.

ACTIONS ON *I_{Kr}* AND *I_{Ks}*

Isoprenaline has complex actions on *I_K*. β-adrenergic agonists have been reported to increase *I_K* in rabbit SAN cells (Lei et al., 2000, 2002), small multi-cellular preparations of rabbit SAN (Walsh et al., 1988), guinea-pig SAN cells (Freeman and Kass, 1993), guinea-pig ventricular myocytes (Yazawa and Kameyama, 1990), and frog atrial cells (Giles et al., 1989). In rabbit SAN cells, *I_K* consists of two components, a rapidly activating *I_{Kr}* and a slowly activating *I_{Ks}*, of which *I_{Kr}* is normally the predominant component (Habuchi et al., 1995; Ito and Ono, 1995; Lei and Brown, 1996; Lei et al., 2000, 2002). ISO has been found to increase the

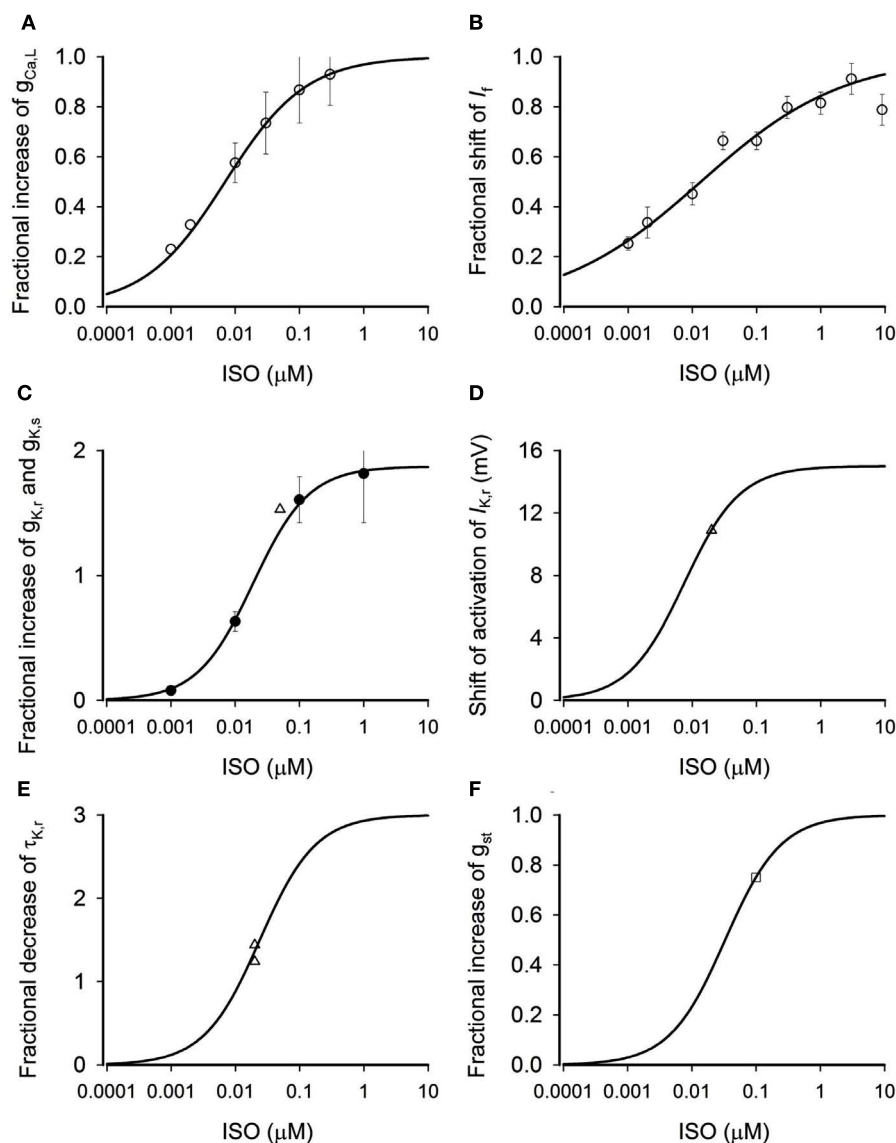


FIGURE 1 | Concentration-dependent effects of ISO. (A)

Dose-dependent fractional increase of g_{CaL} . Open circles: experimental data from Zaza et al. (1996) from rabbit SAN cells. Solid line: computed from model. **(B)** Dose-dependent fractional shift of the I_f activation curve. Open circles: data from Zaza et al. (1996) from rabbit SAN cells. Solid line: computed from model. **(C)** Dose-dependent fractional increase of g_K . Solid circles: data from Yazawa and Kameyama (1990) from guinea-pig SAN cells. Open triangle: data from Lei et al. (2000) from

rabbit SAN cells. Solid line: computed from model. **(D)** Dose-dependent shift of I_{Kr} activation curve. Open triangle: data from Lei et al. (2000) from rabbit SAN cells. Solid line: computed from model. **(E)** Dose-dependent fractional decrease of time constant of deactivation of I_{Kr} . Open triangles: data from Lei et al. (2000) from rabbit SAN cells. Solid line: computed from model. **(F)** Dose-dependent fractional increase of g_{st} . Open square: data from Guo et al. (1995) from rabbit SAN cells. Solid line: computed from model.

current amplitude of I_{Kr} and I_{Ks} (Lei et al., 2000, 2002), increase the rate of deactivation of I_{Kr} in rabbit SAN cells (Lei et al., 2000; Ke et al., 2007), and shift the activation curve of I_{Kr} toward more negative membrane potentials in guinea-pig ventricular myocytes (Yazawa and Kameyama, 1990), frog atrial cells (Giles et al., 1989), and rabbit SAN cells (Lei et al., 2000).

INCREASE OF g_{Kr} AND g_{Ks} (I_{Kr} AND I_{Ks})

The equation for concentration-dependent increase in g_{Kr} was constructed from experimental data of Lei et al. (2000, 2002)

obtained from rabbit SAN cells shown as in **Figure 1C** (triangles) and data of Yazawa and Kameyama (1990) obtained from guinea-pig ventricular myocytes shown as in **Figure 1C** (circles). The equation takes the form:

$$f_K = f_{K,max} \frac{[ISO]}{K_{0.5,gK} + [ISO]} \quad (3)$$

Where f_K is the percentage increase of I_{Kr} . By fitting Eq. 3 to the experimental data of Lei et al. (2000, 2002) and Yazawa and

Kameyama (1990), we obtained the best fit values for $f_{K,\max}$ (the maximum percentage increase I_K) and $K_{0.5,g_{Kr}}$ (the ISO concentration required to half-maximally increase of g_{Kr}) as 1.87 (i.e., 187%) and 19 nM, respectively. The solid line in **Figure 1C** shows the relationship between the increase in g_{Kr} and the ISO concentration predicted by Eq. 3.

Isoprenaline has also been found to increase I_{Ks} : experimental data have shown that 10 nM ISO increased I_{Ks} by about 20% (Lei et al., 2002), which is comparable to the increase of I_{Kr} with a similar ISO concentration. Due both to this similarity and to the lack of availability of complete experimental concentration-response data, in this study we assumed a similar concentration-dependent modulation by ISO of g_{Ks} and g_{Kr} , both of which were modeled by Eq. 3.

SHIFT IN ACTIVATION OF I_{Kr}

The equation to describe the shift in the voltage-dependent activation relation of I_{Kr} (S_K) was constructed from experimental data of Lei et al. (2000) obtained from rabbit SAN cells. As there are not sufficient experimental data available in respect of concentration-dependence of the effect, we assumed that the concentration-dependent shift of the I_{Kr} activation curve is the same as dose-dependent increase of g_{Kr} , which takes the form:

$$S_K = S_{K,\max} \frac{[\text{ISO}]}{K_{0.5,K\text{acti}} + [\text{ISO}]} \quad (4)$$

By fitting Eq. 4 to the experimental data of Lei et al. (2000) as shown in **Figure 1D** (triangle), we obtained the best fit value of $S_{K,\max}$ (the maximal shift of I_K , in mV) as -15 mV and $K_{0.5,K\text{acti}}$ as 7.5 nM.

INCREASE IN THE RATE OF DEACTIVATION OF I_{Kr}

The equation describing the increase in the rate of deactivation of I_{Kr} was constructed from experimental data on I_K from Lei et al. (2000) obtained from rabbit SAN cells, shown in **Figure 1E** (triangles). In the Figure, data are presented as the decrease of the time constant of deactivation (τ_{Kr}). Once again, we assumed that the dose-dependent change of τ_{Kr} is as the same as the dose-dependent increase of g_{Kr} . The equation takes the form:

$$d_{\tau_{Kr}} = d_{\tau_{Kr},\max} \frac{[\text{ISO}]}{K_{0.5,\tau_{Kr}} + [\text{ISO}]} \quad (5)$$

$d_{\tau_{Kr}}$ is the fractional decrease of τ_{Kr} . By fitting the equation to the experimental data of Lei et al. (2000), we obtained the best fit value of $d_{\tau_{Kr},\max}$ as 3.0 and $K_{0.5,\tau_{Kr}}$ as 24 nM.

INCREASE OF I_{st}

Isoprenaline has been reported to increase I_{st} (Guo et al., 1995, 1997; Shinagawa and Noma, 2000; Toyoda et al., 2005). The equation for the increase of I_{st} was constructed from experimental data obtained from rabbit SAN cells (Guo et al., 1997). Due to a lack of availability of sufficient experimental data, we assumed that the concentration-dependent increase of I_{st} is the same as the concentration-dependent increase of g_{Kr} . The equation takes the form:

$$f_{st} = f_{st,\max} \frac{[\text{ISO}]}{K_{0.5,st} + [\text{ISO}]} \quad (6)$$

By fitting Eq. 6 to the experimental data of Guo et al. (1997) as shown in **Figure 1F** (open square), we obtained the best fit value of $f_{st,\max}$ as 1.0 and $K_{0.5,st}$ as 33 nM.

Ca²⁺ HANDLING

Isoprenaline was found to increase the amplitude and minimal diastolic level of $[\text{Ca}^{2+}]_i$ in mammalian (Hagiwara, 1989; Huser et al., 2000; Vinogradova et al., 2002, 2008; Shannon et al., 2004; Maltsev and Lakatta, 2009; Wu et al., 2009) and amphibian (Ju and Allen, 1999) pacemaker cells. It has been found that ISO altered SR Ca²⁺ uptake and release by stimulation of calmodulin kinase II (CamKII; Shannon et al., 2005; Maltsev and Lakatta, 2009). In simulations of the effect of ISO, we adopted the approach of Kharche et al. (2011) to modify the Ca²⁺ handling equations to increase the amplitude and minimal diastolic level of $[\text{Ca}]_i$, as observed in experimental studies (Ju and Allen, 1999; Vinogradova et al., 2002) by increasing the maximal SR Ca²⁺ release (by 20%) and reducing the SR Ca²⁺ release (by 20%) fluxes.

Equations 1–6 were incorporated into the action potential mathematical models of Boyett et al. (2001) and Kurata et al. (2008) for central and peripheral rabbit SAN cells to simulate the chronotropic effect of ISO and to investigate the ionic basis underlying such a chronotropic effect.

RESULTS

CHRONOTROPIC EFFECT OF ISOPRENALINE

The simulated effects of ISO on spontaneous action potentials are shown in **Figure 2**. In the figure, the left-hand panels show the results computed from the central (A) and peripheral (C) Boyett et al. cell models, the right-hand panels show the results computed from the central (B) and peripheral (D) Kurata et al. cell models. For each cell-type and each model, the effects of ISO with a concentration of 0.05 μM are shown. In all cell models, ISO affected the shape of action potentials: it accelerated the firing rate, shortened the action potential duration, increased the amplitude of action potentials, and the maximal diastolic potential (the maximal diastolic potential became more negative). These simulated effects of ISO on action potentials are consistent with experimental observations on rabbit SAN cells (Zaza et al., 1996; Lei et al., 2000).

Effects of ISO on ionic currents underlying action potentials (upper panel in each of **Figure 2**; Left panels for the Boyett et al. central (**Figure 2A**) and peripheral (**Figure 2C**) cell models; and right panels for the Kurata et al. central (**Figure 2B**) and peripheral (**Figure 2D**) of cell model were then studied [namely on I_{CaL} , I_f , I_K (the sum of I_{Kr} and I_{Ks}), and I_{st}]. The profiles of I_{CaL} (the second panel), I_f (the third panel), I_K (the fourth panel), and I_{st} (the fifth panel) are shown in **Figure 2** under control conditions and in the presence of 0.05 μM ISO. In the simulated presence of ISO the inward currents I_{CaL} , I_f , and I_{st} were increased, while net I_K was increased in the repolarization phase but decreased in the diastolic depolarization phase. The increased I_K during action potential repolarization sped up the rate of repolarization (thereby shortening action potential duration) and increased the maximal diastolic potential. Increases of the inward currents I_{CaL} , I_f , and I_{st} , together with a decrease of the outward current I_K in the depolarization phase accelerated the rate of depolarization

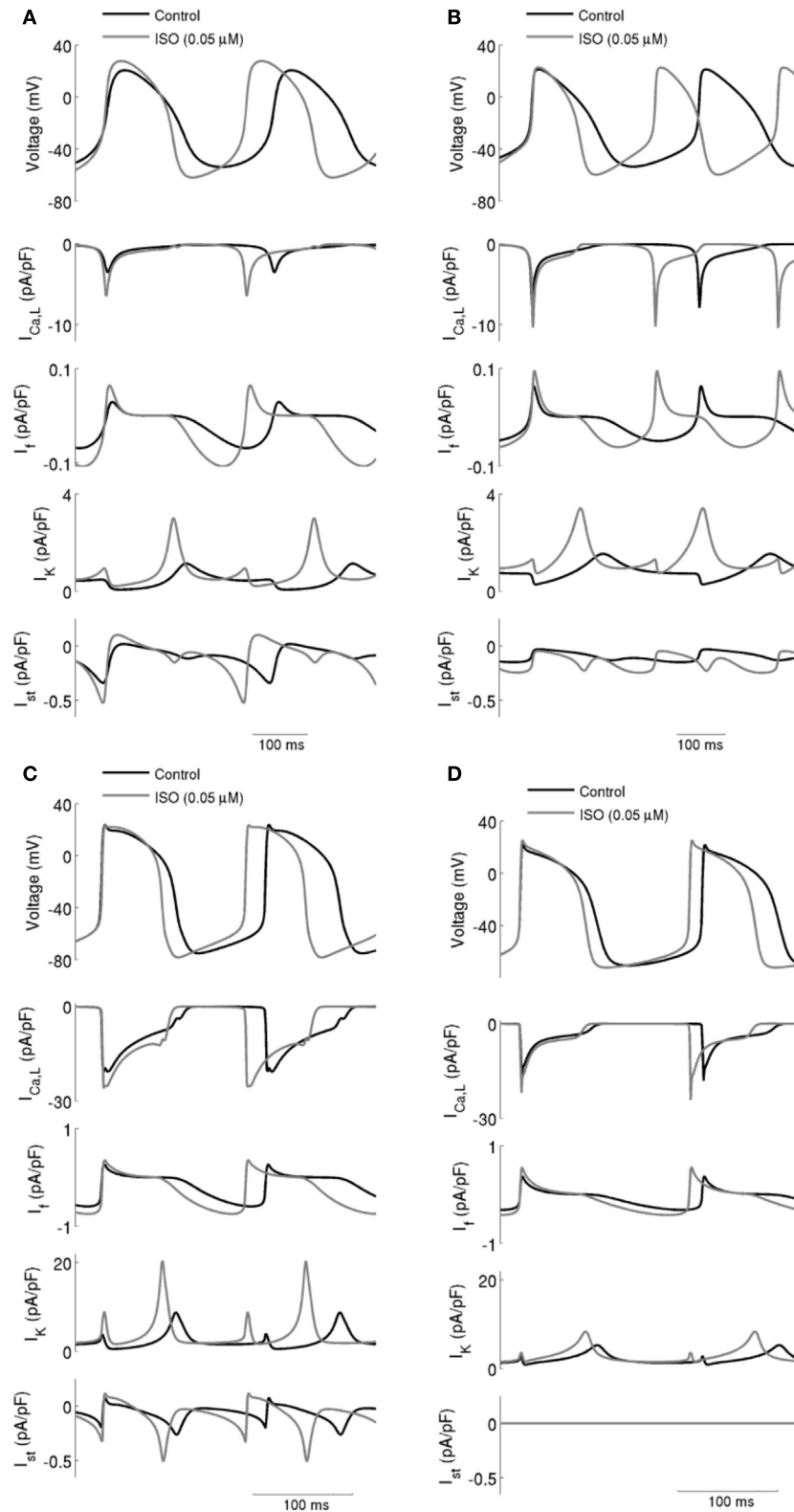


FIGURE 2 | $I_{Ca,L}$, I_f , I_K (sum of I_{K1} and I_{Ks}), and I_{st} during action potentials in control and with ISO. Data in control and with 0.05 μM ISO are shown for: (A) Boyett et al.; (B) Kurata et al. central cell models. (C) Boyett et al.; (D)

Kurata et al. peripheral cell models. In all four cell models, ISO caused an increase in spontaneous firing rate and this was accompanied by increases in $I_{Ca,L}$, I_f , I_K , and I_{st} .

and therefore the rate of the spontaneous activity. The increase in action potential amplitude can be attributed to an increase in I_{CaL} .

The simulated effects of ISO exhibited clear concentration-dependence: the higher the simulated ISO concentrations, the greater the observed chronotropic effect. This is shown in **Figure 3**, in which action potentials computed under control conditions are superimposed on those computed with different ISO concentrations for the central (A) and peripheral (C) Boyett et al. cell models (left-hand panels), and the central (B) and peripheral (D) Kurata et al. (right-hand panels) cell models. In simulations using the Boyett et al. (2001) models, 0.005 μM ISO increased the rate by 7% for the central cell model and by 6% for the peripheral cell model. 0.05 μM ISO increased the rate by 20% for the central cell model and 15% for the peripheral cell model. 0.5 μM ISO increased the rate by 21% for the central cell model and 16% for the peripheral cell model. In simulations using the Kurata et al. (2008) models, 0.005 μM ISO increased the rate by 13% for the central cell model but did not significantly change rate for the peripheral cell model. 0.05 μM ISO increased the rate by 25% for the central cell model and 7% for the peripheral cell model; 0.5 μM ISO increased the rate by 29% for the central cell model and 16% for the peripheral cell model. The results summarizing the simulated chronotropic effects of ISO are shown in **Figure 4**, in which the percentage of decrease of pacemaking cycle length (BCL) is plotted against ISO concentration. The data computed from the central cell models of Boyett et al. and Kurata et al. are shown in **Figures 4A,B**, respectively. The data computed from the peripheral cell models of Boyett et al. and Kurata et al. are shown in **Figures 4C,D**, respectively. The simulated data (solid line) were compared with the experimental data obtained from rabbit isolated SAN cells by Lei et al. (2000; open circle), Zaza et al., 1996; open right-facing triangle), Choi et al. (1999; open diamond), and Barbuti et al. (2007; open inverted triangle). Experimentally, 0.02 μM ISO increased the rate by 13% (Lei et al., 2000), similar to simulated response of all four cell models to the same ISO concentration. The discrepancy between the simulations and the experimental data of Zaza et al. (1996) or the Choi et al. (1999) is slight and could be attributable to model limitations (see Discussion). Note that the simulation data from the central cell models matched better to experimental data than the peripheral cell models as in those experimental studies only primary SAN cells were considered without distinguish between central and peripheral cells. The roles of I_{CaL} , I_f , I_{Kr} , I_{Ks} , and $[\text{Ca}^{2+}]_i$ in the ISO-produced positive chronotropy were also considered, as shown in **Figure 5**.

THE ROLE OF I_{CaL}

The role of I_{CaL} in ISO-induced positive chronotropy was investigated by comparing the effect of ISO when its action on I_{CaL} was incorporated into the models with that observed when this action was removed. The concentration-dependence of the change in rate (when I_{CaL} is not affected by ISO while I_f , I_{Kr} , I_{Ks} , $[\text{Ca}]_i$, and I_{st} are affected by ISO) is shown in **Figure 5** (black dash-dotted line), whilst the standard response [obtained when all actions of ISO were considered (solid black line)] is also shown. In the central cell models, without affecting I_{CaL} , the concentration-dependent change of rate was similar to the standard response. For the peripheral cell models, when I_{CaL} was unaltered by ISO,

the concentration-dependent change of rate was found always to be larger than the standard value in the whole range of ISO concentration considered. This implies that the increase of I_{CaL} did not contribute to the ISO increase of the rate under our simulation conditions; indeed, if anything, it decreased rate.

THE ROLE OF I_f

Isoprenaline shifts the voltage dependence of activation curve of I_f to more positive membrane potentials with a maximal shift of about +15 mV in rabbit SAN cells (Zaza et al., 1996; Accili et al., 1997a,b; and about +7 to +18 mV in murine SAN cells; Alig et al., 2009; Baruscotti et al., 2010; Liao et al., 2010). This shift results in an increase in I_f over voltages relevant to the pacemaker potential range. Adopting an approach similar to that taken above for I_{CaL} , the role of I_f in ISO-induced positive chronotropy was investigated by comparing simulations incorporating the effects of ISO on I_f with those in which this action was not incorporated. The simulated data are shown in **Figure 5** (solid gray line) and compared with the standard simulations (solid black line). In both the central and peripheral cell models the concentration-dependent changes of the rate were reduced compared with the standard value, which suggests I_f contributes to the ISO-induced positive chronotropy.

The computed action potentials and I_f under different conditions are presented in **Figure 6**, in order to illustrate more clearly the role of I_f in the chronotropic effect of ISO. The left-hand panels show the results computed from the Boyett et al. models, whilst the right-hand panels show the results computed from the Kurata et al. models. “control” traces were obtained without simulating effects of ISO, traces labeled “ISO” were obtained from incorporating all actions of 0.05 μM ISO, whilst traces labeled “ISO, no I_f shift” omit actions on I_f . In these simulations, ISO accelerated the pacemaking rate by reducing the cycle length of spontaneous activity for both the central (**Figures 6A,B**) and peripheral (**Figures 6C,D**) cell models. In the case of “ISO, no I_f shift,” the pacemaker activities of both models are slowed down, but by less than 3% compared to the “ISO” condition. The positive shift in voltage-dependent activation for I_f generated a significant increase in the magnitude of I_f as shown in the lower panels in **Figures 6A,B** (central models) and **Figures 6C,D** (peripheral models). However, our simulations indicate that the increase of I_f was not exclusively the result of this shift, as when it was absent there was still an increase in I_f , attributable to the increase of the maximal diastolic potential.

THE ROLE OF I_{Kr}

The role of I_{Kr} in the chronotropic effect of ISO was comprised of three components: the increase of g_{Kr} , the shift of the I_{Kr} activation curve, and the increase of the rate of deactivation of I_{Kr} . By adopting a simulation approach, it was possible to investigate in turn the contribution of each of these elements to the action of ISO. The computed concentration-dependent changes in spontaneous rate of the central and peripheral models when the action of ISO on g_{Kr} was omitted are shown in **Figure 5** (dashed gray line) and compared with the standard ISO simulation (solid black line). In these simulations, the Boyett et al. and the Kurata et al. central cell models responded in a qualitatively similar fashion. In both Boyett et al. and Kurata et al. central SAN models (**Figures 5A,B**), removing the ISO action on g_{Kr}

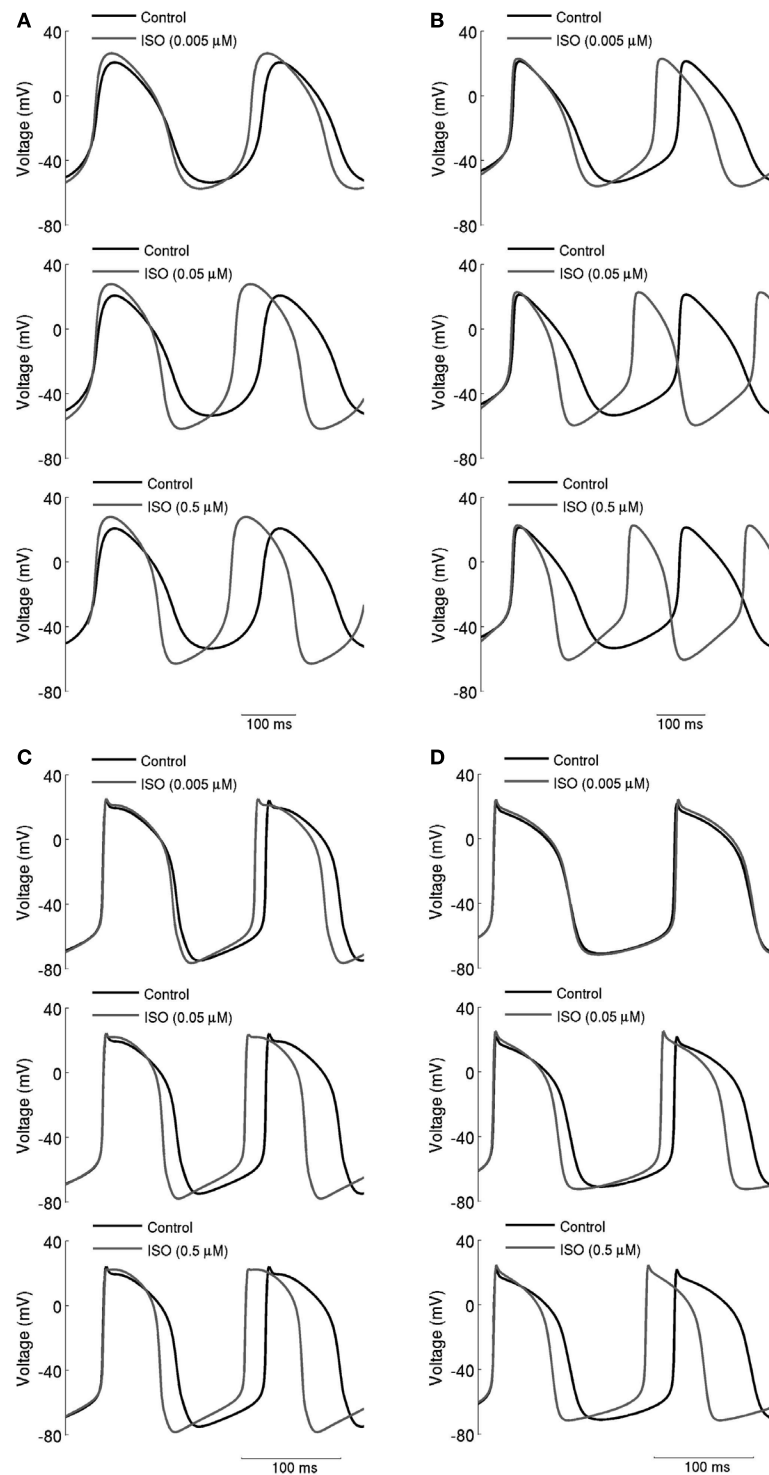


FIGURE 3 | Concentration-dependent effects of ISO on action potentials. (A) Boyett et al.; **(B)** Kurata et al. central cell models. **(C)** Boyett et al.; **(D)** Kurata et al. peripheral cell models. Action potentials are

shown under control conditions and in the presence 0.005, 0.05, and 0.5 μM ISO. Spontaneous firing rate was increased by ISO in a dose-dependent manner.

accelerated pacemaking as demonstrated by a greater decrease in pacemaking cycle length as compared to the standard condition. Such an enhanced chronotropic effect without ISO modification

of g_{K_r} was also observed in the Boyett et al. peripheral cell model (**Figure 5C**), though data from the Kurata et al. peripheral cell model (**Figure 5D**) showed a different response. In the Kurata et

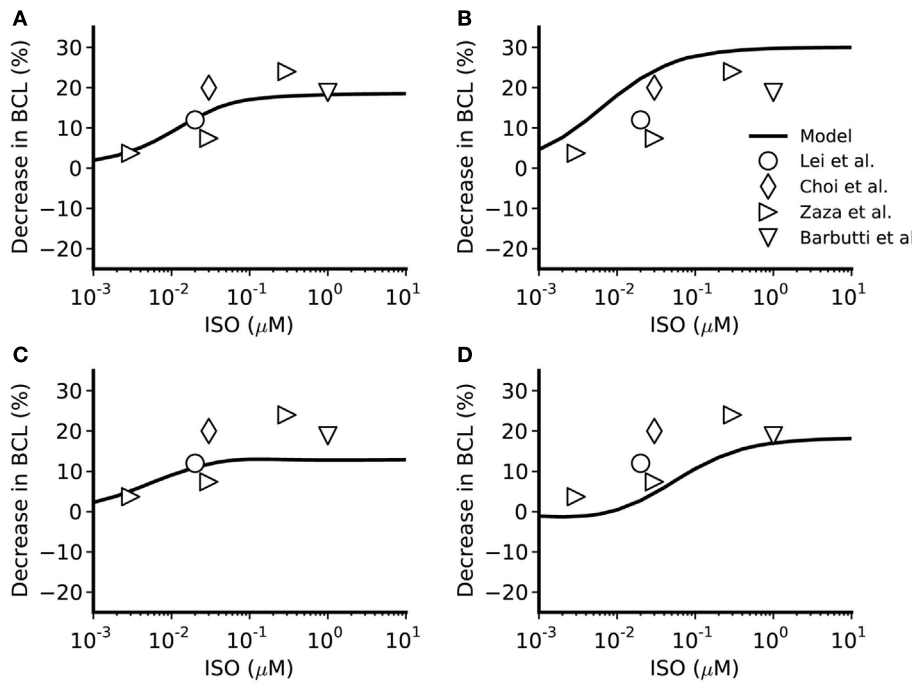


FIGURE 4 | Concentration-dependent percentage decrease of pacemaking cycle length (BCL) by ISO. (A,B) Boyett et al. and Kurata et al. central cell models. **(C,D)** Boyett et al. and Kurata et al. peripheral cell models. The figure incorporates concentration-response data from the models and relevant experimental data. Black line: data from the standard configuration of

model in which effects of ISO are incorporated for all of I_{CaL} , I_t , I_{Kr} , I_{Ks} , I_{str} , and Ca^{2+} transient. Open circles: experimental data of Lei et al. (2000) from rabbit SAN cells. Open right-facing triangle: data of Zaza et al. (1996) from rabbit SAN cells. Open diamonds: data of Choi et al. (1999) from rabbit SAN cells. Open inverted triangles: data of Barbutti et al. (2007) from rabbit SAN cells.

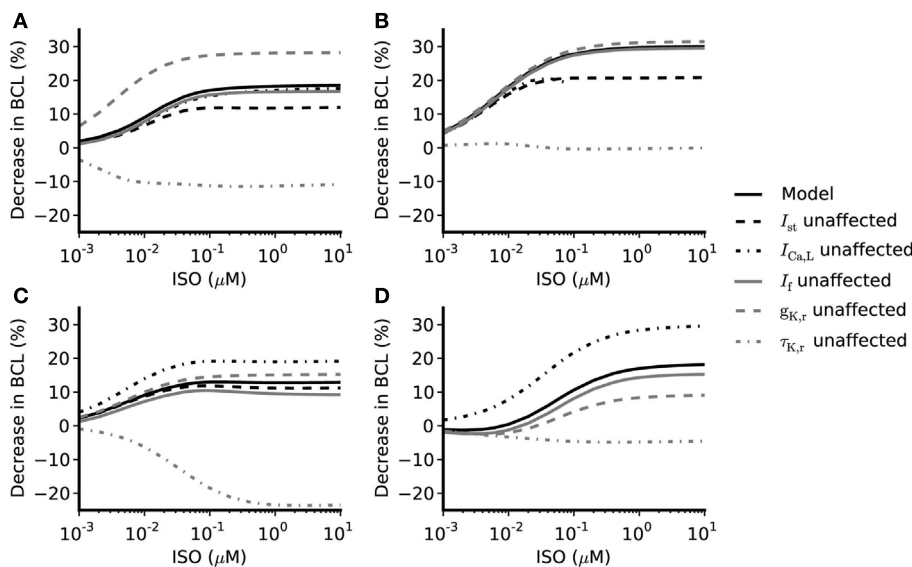


FIGURE 5 | Concentration-dependent percentage decrease of pacemaking cycle length (BCL) by ISO under different simulation conditions. (A,B) Boyett et al. and Kurata et al. central cell models **(C,D)** Boyett et al. and Kurata et al. peripheral cell models. Black solid line: data from the standard configuration of model in which effects of ISO are incorporated for all of I_{CaL} , I_t , I_{Kr} , I_{Ks} , I_{str} , and Ca^{2+} transient. Black

dash-dotted line: data from model when effects on I_{CaL} omitted. Gray solid line: data from model when effects on I_t omitted. Gray dashed line: data from model when g_{Kr} not affected by ISO. Gray dash-dotted line: data from model when time constant of deactivation of τ_{Kr} not affected by ISO. Black dashed line: data from model when effects of ISO on I_{st} omitted.

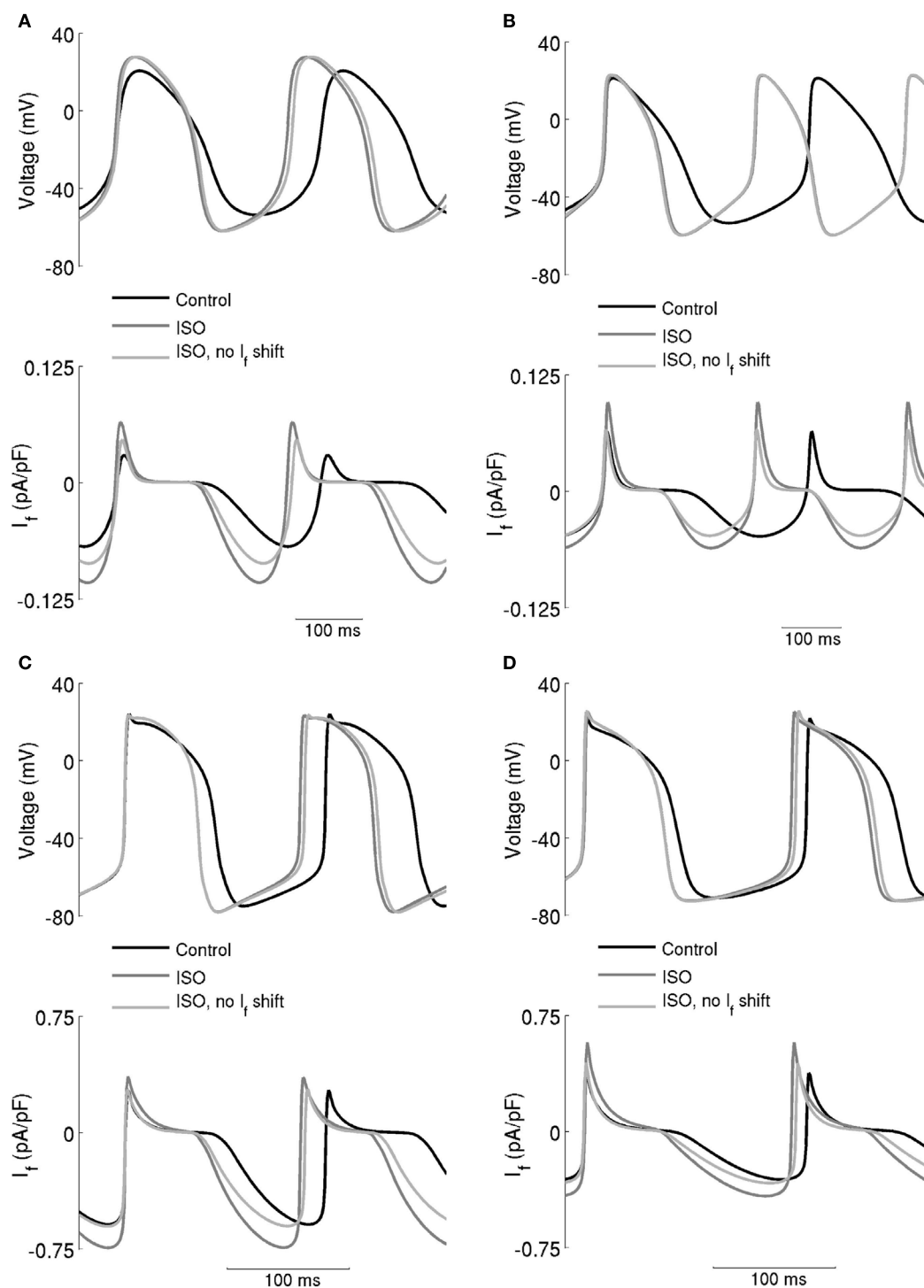


FIGURE 6 | Effect of the ISO-induced increase of I_f on spontaneous activity. (A) Boyett et al. and **(B)** Kurata et al. central cell models. **(C)** Boyett et al. and **(D)** Kurata et al. cell models. Each panel shows action potentials (upper traces) and records of I_f (lower

traces) under control conditions and in the presence of $0.05 \mu\text{M}$ ISO. Two simulations with ISO are shown – in one, I_f was affected and in the other it was not. This shows that the role of I_f in the chronotropic effect of ISO is small.

al. peripheral cell model, removing the ISO action on g_{K_r} resulted in a reduced decrease in the pacemaking cycle length as compared to the standard value.

In simulations, we also computed concentration-dependent change in spontaneous rate when the action of ISO on voltage dependence of activation of I_{K_r} was omitted, and compared the

results with the standard value (data not shown). In both the Boyett et al. and Kurata et al. central cell models, the computed values are larger than the standard values, which suggests that the shift in voltage-dependent activation of I_{Kr} does not contribute to ISO-stimulated increase in pacemaking rate (in contrast, it appeared actually to reduce pacemaking rate). Omitting this action of ISO had a negligible effect on the response of the peripheral cell models. Similarly, the concentration-dependent change of the pacemaking rate when the action of ISO on τ_{Kr} was omitted is also shown in **Figure 5** (gray dash-dotted line). In both central and peripheral cell models, removal of the action of ISO on τ_{Kr} had a dramatic influence of the chronotropic effect of ISO. In the central cell models, when effects on τ_{Kr} were omitted ISO decreased the pacemaking rate (more so in Boyett et al. model, compared to that of Kurata et al.). Similar effects were seen for the peripheral cell models, for which ISO significantly slowed down the pacemaking rate at high concentrations. The results of these simulations indicate that the effect of ISO on τ_{Kr} plays an important role in the chronotropic effect of ISO.

The underlying basis for the influence on spontaneous rate of τ_{Kr} modification by ISO is shown in **Figure 7**. This shows the action potentials for Boyett et al. central (A) and peripheral (C) cell models (left-hand panels), and the Kurata et al. central cell (B) and peripheral (D) cell models (right-hand panels). The traces labeled “control” were obtained in the absence of ISO, the traces labeled “ISO” were obtained from simulations in which all effects of $0.05 \mu\text{M}$ ISO were considered. The traces labeled “ τ_{Kr} affected only” were obtained from simulations in which only the effect of $0.05 \mu\text{M}$ ISO on “ τ_{Kr} ” was considered. Also shown in the figure, in the lower panels, are the corresponding I_{Kr} records. In the Boyett et al. model simulations, $0.05 \mu\text{M}$ ISO increased the pacemaking rate by 20 and 15% for the central and peripheral cell models, respectively. In both models, ISO increased the maximal diastolic potential (i.e., become more negative; see **Table 1**). However, when only τ_{Kr} was affected, ISO increased the rate by 33 and 10% for the central and peripheral cell models, respectively, each with an elevated maximal diastolic potential (see **Table 1**). Compared with the “control” condition, in both central and peripheral cell models ISO increased I_{Kr} during the initial repolarization phase, but reduced I_{Kr} in later repolarization and the depolarization phase. These changes in I_{Kr} result from altered τ_{Kr} . When τ_{Kr} only was affected, an increase of I_{Kr} in the early repolarization phase was observed, whilst a decrease of I_{Kr} in late repolarization period and subsequent depolarization phase was seen. The decreased I_{Kr} during in the depolarization phase contributed significantly to the increase of the pacemaking rate. Similar observations were seen with the Kurata et al. models (**Table 1**). $0.05 \mu\text{M}$ ISO increased the pacemaking rate by 25% for the central cell model and 7% for the peripheral cell model. When τ_{Kr} only was affected, ISO increased the rate by 20% for the central cell model and by 9% for the peripheral cell model.

In simulations, we also computed concentration-dependent change in spontaneous rate when the action of ISO on increasing g_{Ks} was omitted. As compared to the results obtained in the standard condition, omitting ISO action on g_{Ks} produced negligible effect on the pacemaking rate (data not shown).

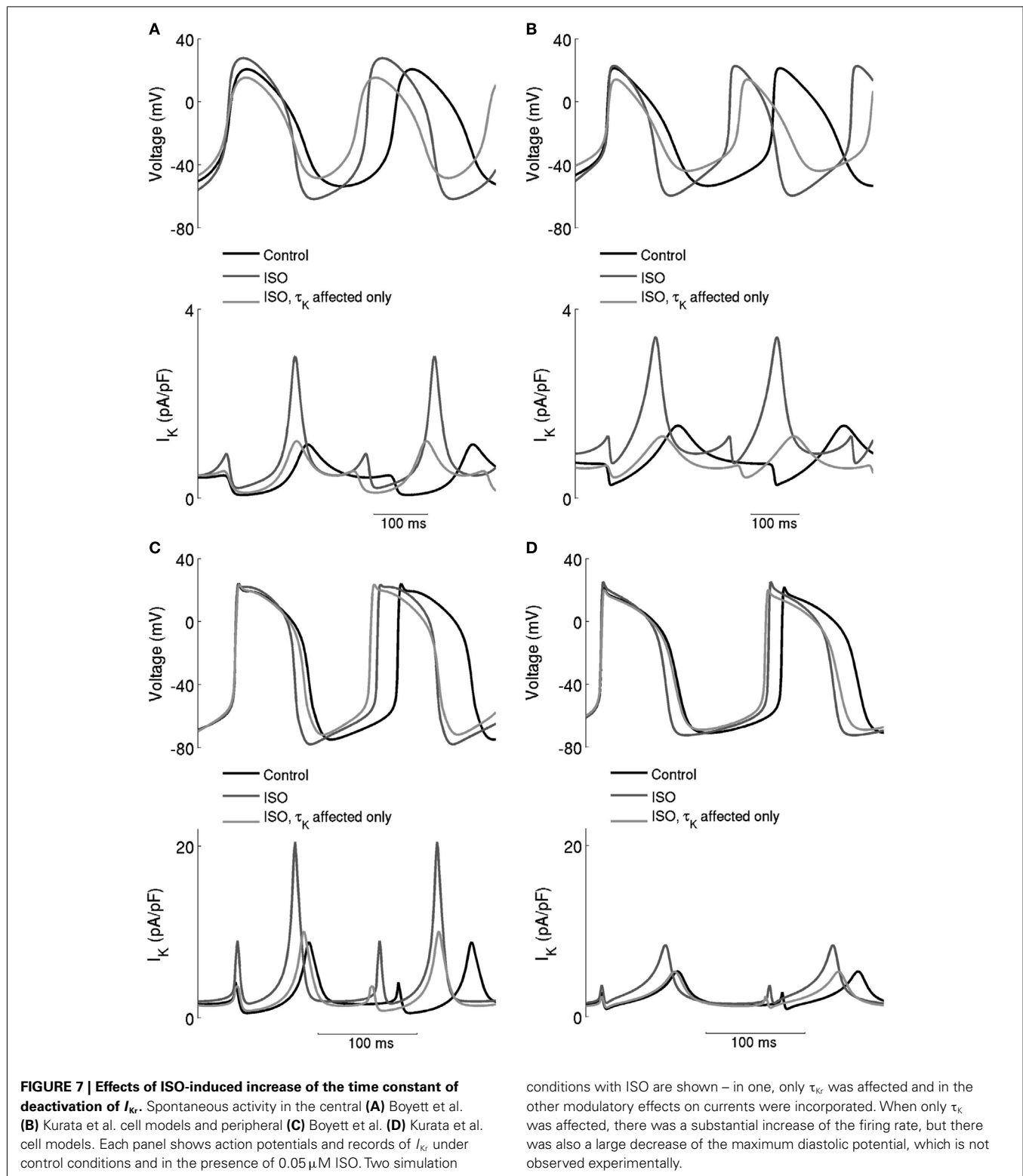
In a previous experimental study, Lei et al. (2002) have shown that blocking I_{Ks} produced a negligible effect on spontaneous rate of rabbit SAN cells under control condition. However, with application of 10 nM ISO, blocking I_{Ks} produced $\sim 10\%$ prolongation of pacemaking cycle length. This implies that I_{Ks} plays a more important role in generating SAN pacemaking with ISO present than in its absence (Lei et al., 2002). This experimental observation on the role of I_{Ks} in the pacemaking potentials of rabbit SAN cells was partially reproduced by the model. In simulations, blocking I_{Ks} in the control condition produced negligible alteration to the pacemaking cycle length in both of the Boyett et al. central and peripheral cell models. With 10 nM ISO, blocking I_{Ks} produced an increase of ~ 3 and 2% in the pacemaking cycle length for the central and peripheral cell model, respectively. These simulation results are qualitatively similar to the experimental observations of Lei et al. (2002). Quantitative differences between the model simulation and the experimental data of Lei et al. (2002) on the role of I_{Ks} in SAN cell activity under control and ISO conditions may be due to the intrinsic limitations of the model as discussed in detail in Zhang et al. (2000), or due to limitations in simulating ISO (see Discussion).

THE ROLE OF I_{st}

Similar to other currents investigated, the role of I_{st} in the positive chronotropic effect of ISO was investigated by comparing simulations of the effect of ISO that incorporated its effects on I_{st} with those in which this action was omitted. The computed data are shown in **Figure 5** (black dashed line) and can be compared with the standard response incorporating all effects of ISO (solid black line). By removing the action of ISO on I_{st} , the computed dose-dependent changes in the Boyett et al. central cell model were only slightly reduced compared with the standard values. In the Boyett et al. peripheral cell model, the computed concentration-dependent changes of rate were also close to the standard values. These results suggest that I_{st} does not play an important role in ISO-induced positive chronotropy under our simulation conditions. As there is no I_{st} in the Kurata et al. cell models, the role of augmented I_{st} in the positive chronotropic effect of ISO was not analyzed using the Kurata et al. cell models.

THE ROLE OF Ca^{2+} HANDLING AND Na^{+} - Ca^{2+} EXCHANGE

Experimental studies have shown that that ISO increases the systolic and diastolic levels of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) (Ju and Allen, 1999; Vinogradova et al., 2002, 2008). Here we considered how ISO-induced changes in I_{NaCa} consequent upon ISO-induced changes in $[\text{Ca}^{2+}]_i$ contribute to the positive chronotropic action of ISO, when effects on other currents are excluded. The results from Boyett et al. models are shown in **Figure 8**. In the figure the time traces of action potentials (**Figures 8Ai,Bi**), $[\text{Ca}^{2+}]_i$ (**Figures 8Aii,Bii**), and I_{NaCa} (**Figures 8Aiii,Biii**) were superimposed in different conditions. The traces labeled “control” were obtained in the absence of ISO, the traces labeled “ISO” were obtained from simulations in which the ISO-induced changes in systolic and diastolic $[\text{Ca}^{2+}]_i$ alone were considered. In both the central and peripheral cell models, the systolic and diastolic levels of $[\text{Ca}^{2+}]_i$ were doubled by ISO. In simulations using the Boyett et al. models, changes in $[\text{Ca}^{2+}]_i$ increased the rate by 10% for the



central model, and 1% for the peripheral cell model. The accelerated pacemaking rate was attributable to an increased I_{NaCa} during the diastolic pacemaking phase (Figures 8Aiii, Biii) that arose from an elevated diastolic $[Ca^{2+}]_i$ level due to an integral action of an

increased the SR Ca^{2+} release and reduced SR Ca^{2+} uptake. Further simulations were also performed to investigate the individual role of an increased SR Ca^{2+} release and a reduced SR Ca^{2+} uptake. By either increasing the SR Ca^{2+} release alone or reducing the SR

Table 1 | Effects of ISO and ISO-induced change in the deactivation rate of I_{Kr} on the characteristics of pacemaking action potentials.

		Boyett et al. model		Kurata et al. model	
		Central	Peripheral	Central	Peripheral
ISO (0.05 mM); all actions considered	Δ Rate	20%	15%	25%	7%
	Δ MDP	-10 mV	-2 mV	-6 mV	-2 mV
ISO (0.05 mM); action on τ_{Kr} alone considered	Δ Rate	33%	10%	20%	9%
	Δ MDP	+22 mV	+4 mV	+9 mV	+2 mV

Ca^{2+} uptake alone, the diastolic $[Ca^{2+}]_i$ level was elevated, resulting in an increased I_{NaCa} leading to accelerated pacemaking rates. Results from Kurata et al. cell models were qualitatively similar to the results from the Boyett et al. models.

DISCUSSION

In this study, we have incorporated a set of equations of the known actions of ISO on I_{CaL} , I_f , I_{Kr} and I_{Ks} , I_{st} , and intracellular Ca^{2+} handling into two established action potential models of central and peripheral rabbit SAN cells to simulate the chronotropic effect of ISO. Use of these models has enabled us to manipulate the actions of ISO and dissect out the relative contributions of different ionic currents to the modulatory actions of β -adrenergic stimulation.

The simulated effect of ISO on the spontaneous activity in the SAN cell models studied here is very similar to that seen experimentally in rabbit SAN cells (Zaza et al., 1996; Lei et al., 2000, 2002). ISO accelerated the pacemaking rate and affected the shape of action potentials in both models used here. The computed concentration-dependent increases of the rate are comparable to experimental data obtained from rabbit SAN (Zaza et al., 1996; Lei et al., 2000). With both Boyett et al. (2001) and Kurata et al. (2008) models, ISO increases the overshoot and maximal diastolic potential, and shortens the duration of action potentials. These changes in action potential shape are consistent with experimental observations (Zaza et al., 1996; Lei et al., 2000).

In our simulations, we were able to show that an increase in I_{CaL} contributes to the increase of the overshoot of action potential with ISO, however it contributes very little to the acceleration of firing rate. In the peripheral cell model, when the action of increasing I_{CaL} alone is considered, the firing rate was actually slowed down. Consistent with this, blocking I_{CaL} by nifedipine has been found to increase the pacemaking rate experimentally in small balls cut from the periphery of the rabbit SAN (Kodama et al., 1997). Presumably, the underlying mechanism is related to the effect of increased I_{CaL} on action potential duration, whereby an increase of I_{CaL} leads to an increase in the time interval between two successive action potentials.

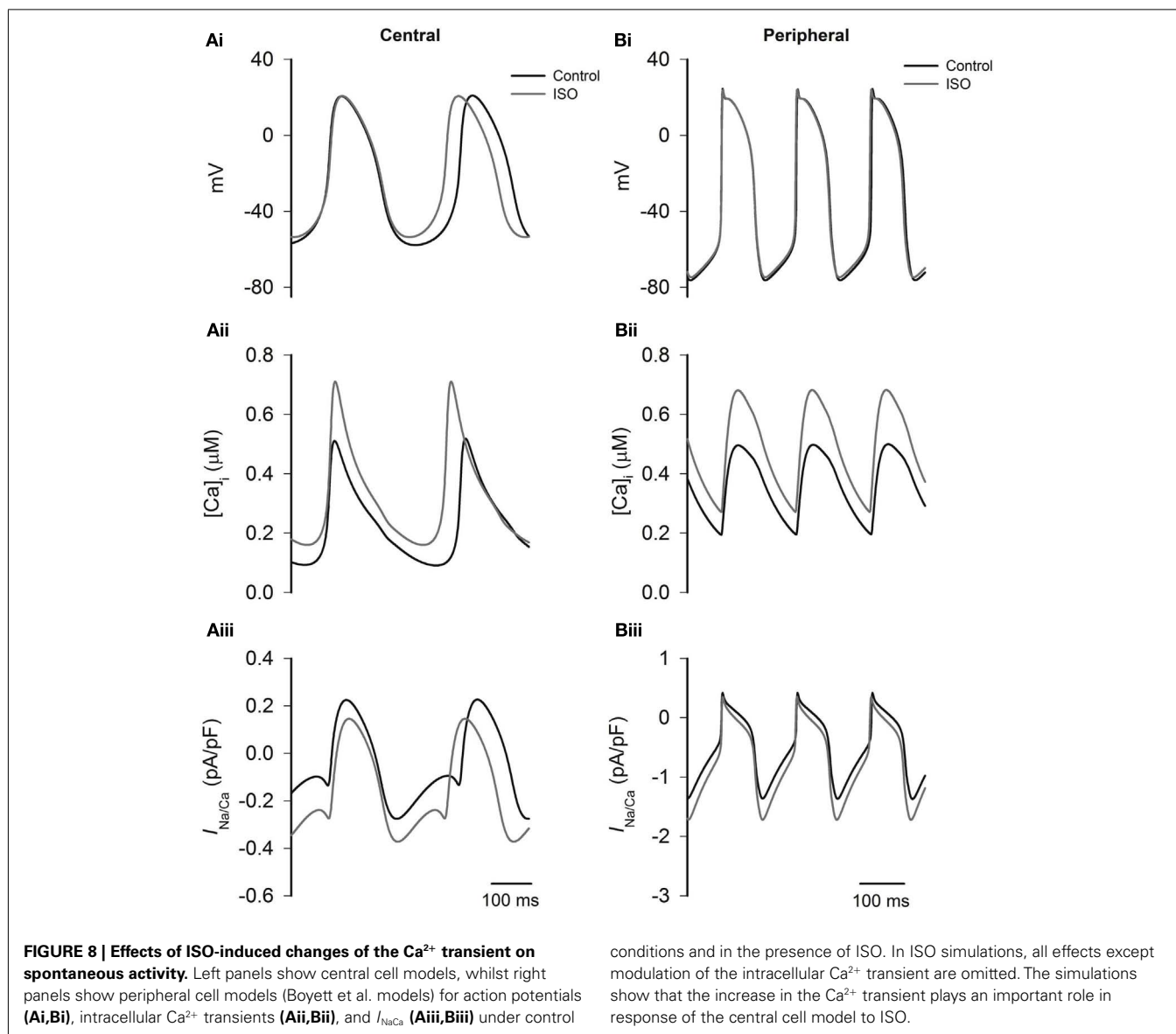
The role of I_f in the chronotropic effect of ISO has been controversial. I_f has been considered by some as the main pacemaking current in rabbit SAN (DiFrancesco, 1995, 2010; DiFrancesco and Borer, 2007) and increased I_f has been considered as the main ionic basis of the chronotropic effect of ISO (Zaza et al., 1996; Liao et al., 2010). However, the role of I_f in β -adrenoceptor mediated chronotropy in the SAN is not unequivocal: Cai et al. (1995) have shown that the positive chronotropic effect of ISO was not

significantly affected when I_f is blocked by Cs^+ . Also, in the amphibian sinus venosus, in which there is no I_f , ISO still accelerated the firing rate (Ju and Allen, 1999), whilst mice lacking the predominant I_f channel gene (HCN4) exhibit physiological heart rate responses to isoproterenol (Herrmann et al., 2007). In our simulations, we have observed that I_f does contribute to the chronotropic effect of ISO but that its contribution is small, as removal of the action of ISO on I_f produces a small alteration to the simulated concentration-response relation for the rate increase.

The role of I_{st} in the chronotropy was also considered. In both models removal of the action of ISO on I_{st} does not affect the dose-dependent increase of rate significantly. In simulations, removal of the action of ISO had a relatively larger effect on the chronotropy in the central cell model than in the peripheral cell model. This is because in the development of the models, due to the absence of experimental data about the regional difference of I_{st} in rabbit SAN, the same magnitude (current density) of I_{st} was assumed for both central and peripheral cell models. Consequently the central cell models were relatively more sensitive to I_{st} , due to relatively smaller current densities of other depolarizing channel currents, such as I_f , I_{Na} , and I_{CaT} , when compared to peripheral cell models (Zhang et al., 2000).

In this study, we have shown that an increased $[Ca^{2+}]_i$ level in the simulated presence of ISO contributed to the increase in rate by reducing the pacemaking cycle length in the central cell model. This observation is qualitatively similar to the results of Maltsev and Lakatta (2009), which provide evidence that the “ Ca^{2+} clock” plays an important role in generating sinoatrial nodal pacemaking. Quantitative differences between our results and those of Maltsev and Lakatta (2009) on the “ Ca^{2+} clock” contribution to pacemaking are likely to reflect differences in the parameterization of the various currents involved in the membrane clock between the Boyett et al. (2001) model and the Maltsev and Lakatta (2009) model. The parameters for ionic channel currents in the Boyett et al. (2001) are inherited from the Zhang et al. (2000) cell models, which were based on and validated against experimental data (Zhang et al., 2000). The difference in the response to raised $[Ca^{2+}]_i$ between the central and peripheral Boyett et al. (2001) models also reflected the experimentally observed intrinsic difference in the current densities of some depolarizing currents between two different cell types, as summarized in Boyett et al. (2000) and Zhang et al. (2000).

The chronotropic effect of ISO reflects the combined actions of ISO on ionic currents I_{CaL} , I_f , I_{Kr} , I_{Ks} , and I_{st} , among which action on I_{Kr} plays a relatively more important role. Removal of the action of ISO on the rate of deactivation of I_{Kr} reduced the chronotropic



effect of ISO significantly in both Boyett et al. (2001) and Kurata et al. (2008) models, and even reverses the chronotropy of ISO with high concentration in the peripheral model. An increase in the rate of deactivation of I_{Kr} increased the magnitude of I_{Kr} in the early action potential repolarization phase, but decreased the magnitude of I_{Kr} in the late repolarization phase and the depolarization phase. A decrease in outward I_{Kr} in the depolarization period enables the net inward current to be relatively larger, which speeds up the depolarization and thus the pacemaking rate.

The important contribution of I_{Kr} to cardiac pacemaking activities has been noted in previous experimental studies. In their work Sato et al. (2000) showed that blocking I_{Kr} partially by ibutilide (though ibutilide is not a completely selective I_{Kr} channel blocker) slowed down rabbit SAN pacemaking rate modestly, but blocking I_{Kr} completely at a higher concentration abolished its pacemaking action potentials. Ono and Ito (1995) also noted the important role of E-4031 sensitive I_{Kr} in rabbit SAN cardiac pacemaking.

Further studies demonstrated that partial block of I_{Kr} (around 50%) by E-4031 almost abolished spontaneous pacemaking activity in central rabbit SAN tissue, though the pacemaking activity in peripheral SAN tissue persisted (Kodama et al., 1999). Data from mouse SAN cells also showed an important contribution of I_{Kr} to cardiac pacemaking action potentials as block I_{Kr} by E-4031 prolonged the pacemaking cycle length by about 68% (Nikmaram et al., 2008). Our simulation data added to these experimental data in showing an important role of I_{Kr} in SAN normal pacemaking, especially in elucidating the role of altered deactivation rate of I_{Kr} in cardiac pacemaking action potentials, which is not easily possible to get from a pharmacological study.

ASSUMPTIONS, LIMITATIONS, AND CONCLUSION

In the present study, due to the lack of available experimental data, we assumed that the concentration-dependent action of ISO on the current amplitude of sinoatrial I_{Ks} and I_{St} was the same as that

of I_{Kr} . This assumption requires to be re-evaluated when more experimental data become available. In addition, where data were not available from rabbit SAN cells, data from other species were used (e.g., some equation parameters for I_{st} were based on multiple data sources from guinea-pig and rat SAN cells (Guo et al., 1995, 1997; Shinagawa and Noma, 2000; Toyoda et al., 2005). Intracellular Ca^{2+} cycling was found to modulate pacemaking rate in the present model, but not as prominently as in a prior simulation study of Maltsev and Lakatta (2009). As the role of the Ca^{2+} clock in SAN pacemaking is debated (e.g., Honjo et al., 2003; Lancaster et al., 2004; DiFrancesco and Borer, 2007; Herrmann et al., 2007; Joung et al., 2009; DiFrancesco, 2010; Gao et al., 2010; Himeno et al., 2011; Sosunov and Anyukhovskiy, 2012), further experimental quantification of SAN cellular intracellular Ca^{2+} handling mechanisms and their contribution to electrogenesis would be useful. Also due to the limited availability of experimental data on rabbit SAN cells, simulations of concentration-dependent ISO action on I_f were based on the experimental data of Zaza et al. (1996), which showed a maximal shift of the steady-state activation curve by +9.62 mV. Though this is relatively smaller as compared to the ISO-induced shift (about +7 to +18 mV) of the I_f activation curve in murine SAN cells (Alig et al., 2009; Baruscotti et al., 2010;

Liao et al., 2010), it is close to observed values of 8.8 mV (Accili et al., 1997a) and 5.3 mV (Accili et al., 1997b) for a 1 μ M ISO-induced shift in voltage-dependent activation of I_f in adult rabbit SAN cells. As there are no experimental data to show other ISO-induced changes in the kinetics of I_f , in simulations, the slope of the steady-state activation curve and the voltage-dependent time constant of activation process were assumed to be unchanged in the ISO condition.

Whilst it is important that potential limitations of the models used in this investigation are made explicit, it is still likely that the findings of our study are valid in showing that the chronotropic effect of ISO involves an integrated action of ISO on I_{CaL} , I_f , I_{st} , I_{Kr} , I_{Ks} , and $[Ca^{2+}]_i$ in order to match prior experimental data of Zaza et al. (1996) and Lei et al. (2000). Our study is significant in highlighting the relative contributions of I_f , Ca^{2+} handling, and modulation of I_{Kr} deactivation kinetics to the overall response to ISO.

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REFERENCES

- Abramochkin, D. V., Kuzmin, V. S., Sukhova, G. S., and Rosenshtraukh, L. V. (2009). Modulation of rabbit sinoatrial node activation sequence by acetylcholine and isoproterenol investigated with optical mapping technique. *Acta Physiol. (Oxf.)* 196, 385–394.
- Accili, E. A., Redaelli, G., and DiFrancesco, D. (1997a). Differential control of the hyperpolarization-activated current ($i(f)$) by cAMP gating and phosphatase inhibition in rabbit sino-atrial node myocytes. *J. Physiol. (Lond.)* 500(Pt 3), 643–651.
- Accili, E. A., Robinson, R. B., and DiFrancesco, D. (1997b). Properties and modulation of I_f in newborn versus adult cardiac SA node. *Am. J. Physiol.* 272(3 Pt 2), H1549–H1552.
- Alig, J., Marger, L., Mesirca, P., Ehmke, H., Mangoni, M. E., and Isbrandt, D. (2009). Control of heart rate by cAMP sensitivity of HCN channels. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12189–12194.
- Barbuti, A., Terragni, B., Brioschi, C., and DiFrancesco, D. (2007). Localization of f-channels to caveolae mediates specific beta2-adrenergic receptor modulation of rate in sinoatrial myocytes. *J. Mol. Cell. Cardiol.* 42, 71–78.
- Baruscotti, M., Bucchi, A., Viscomi, C., Mandelli, G., Consalez, G., Gneschi-Rusconi, T., Montano, N., Casali, K. R., Micheloni, S., Barbuti, A., and DiFrancesco, D. (2010). Deep bradycardia and heart block caused by inducible cardiac-specific knockout of the pacemaker channel gene *Hcn4*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1705–1710.
- Boyett, M. R., Honjo, H., and Kodama, I. (2000). The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc. Res.* 47, 658–687.
- Boyett, M. R., Zhang, H., Garny, A., and Holden, A. V. (2001). Control of the pacemaker activity by the intracellular Ca^{2+} : experiments and simulation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1091–1110.
- Brown, H. F., DiFrancesco, D., and Noble, S. J. (1979). How does adrenaline accelerate the heart? *Nature* 280, 235–236.
- Bucchi, A., Baruscotti, M., Robinson, R. B., and DiFrancesco, D. (2003). $I(f)$ -dependent modulation of pacemaker rate mediated by cAMP in the presence of ryanodine in rabbit sinoatrial node cells. *J. Mol. Cell. Cardiol.* 35, 905–913.
- Cai, Q., Lei, M., and Brown, H. F. (1995). Responses of guinea-pig SA node/atria to acetylcholine and adrenaline in the presence of blockers of i_f and $i_{K,ACH}$. *J. Physiol. (Lond.)* 483, 21.
- Choi, H. S., Wang, D. Y., Noble, D., and Lee, C. O. (1999). Effect of isoprenaline, carbachol, and Cs^+ on Na^+ activity and pacemaker potential in rabbit SA node cells. *Am. J. Physiol.* 276(1 Pt 2), H205–H214.
- DiFrancesco, D. (1995). The onset and autonomic regulation of cardiac pacemaker activity: relevance of the f current. *Cardiovasc. Res.* 29, 449–456.
- DiFrancesco, D. (2010). The role of the funny current in pacemaker activity. *Circ. Res.* 106, 434–446.
- DiFrancesco, D., and Borer, J. S. (2007). The funny current: cellular basis for the control of heart rate. *Drugs* 67(Suppl. 2), 15–24.
- Duchatelle-Gourdon, I., Hartzell, H. C., and Lagrutta, A. A. (1989). Modulation of the delayed rectifier potassium current in frog cardiomyocytes by beta-adrenergic agonists and magnesium. *J. Physiol. (Lond.)* 415, 251–274.
- Freeman, L. C., and Kass, R. S. (1993). Delayed rectifier potassium channels in ventricle and sinoatrial node of the guinea pig: molecular and regulatory properties. *Cardiovasc. Drugs Ther.* 7(Suppl. 3), 627–635.
- Gao, Z., Chen, B., Joiner, M. L., Wu, Y., Guan, X., Koval, O. M., Chaudhary, A. K., Cunha, S. R., Mohler, P. J., Martins, J. B., Song, L. S., and Anderson, M. E. (2010). $I(f)$ and SR Ca^{2+} release both contribute to pacemaker activity in canine sinoatrial node cells. *J. Mol. Cell. Cardiol.* 49, 33–40.
- Giles, W., Nakajima, T., Ono, K., and Shibata, E. F. (1989). Modulation of the delayed rectifier K^+ current by isoprenaline in bull-frog atrial myocytes. *J. Physiol. (Lond.)* 415, 233–249.
- Guo, J., Mitsuiye, T., and Noma, A. (1997). The sustained inward current in sino-atrial node cells of guinea-pig heart. *Pflugers Arch.* 433, 390–396.
- Guo, J., Ono, K., and Noma, A. (1995). A sustained inward current activated at the diastolic potential range in rabbit sino-atrial node cells. *J. Physiol. (Lond.)* 483(Pt 1), 1–13.
- Habuchi, Y., Han, X., and Giles, W. R. (1995). Comparison of the hyperpolarisation-activated and delayed rectifier currents in rabbit atrioventricular node and sinoatrial node. *Heart Vessels Suppl.* 9, 203–206.
- Hagiwara, N. I. H. (1989). Modulation by intracellular Ca^{2+} of the hyperpolarisation-activated inward currents in rabbit single sino-atrial node cells. *J. Physiol. (Lond.)* 409, 121–141.
- Herrmann, S., Stieber, J., Stockl, G., Hofmann, F., and Ludwig, A. (2007). HCN4 provides a “depolarization reserve” and is not required for heart rate acceleration in mice. *EMBO J.* 26, 4423–4432.
- Himeno, Y., Toyoda, F., Satoh, H., Amano, A., Cha, C. Y., Matsuura, H., and Noma, A. (2011). Minor contribution of cytosolic Ca^{2+} transients to the pacemaker rhythm in guinea pig sinoatrial node cells. *Am. J. Physiol. Heart Circ. Physiol.* 300, H251–H261.
- Honjo, H., Inada, S., Lancaster, M. K., Yamamoto, M., Niwa, R., Jones, S. A., Shibata, N., Mitsui, K.,

- Horiuchi, T., Kamiya, K., Kodama, I., and Boyett, M. R. (2003). Sarcoplasmic reticulum Ca^{2+} release is not a dominating factor in sinoatrial node pacemaker activity. *Circ. Res.* 92, e41–e44.
- Huser, J., Blatter, L. A., and Lipsius, S. L. (2000). Intracellular Ca^{2+} release contributes to automaticity in cat atrial pacemaker cells. *J. Physiol. (Lond.)* 524(Pt 2), 415–422.
- Ito, H., and Ono, K. (1995). A rapidly activating delayed rectifier K^+ channel in rabbit sinoatrial node cells. *Am. J. Physiol.* 269(2 Pt 2), H443–H452.
- Joung, B., Tang, L., Maruyama, M., Han, S., Chen, Z., Stucky, M., Jones, L. R., Fishbein, M. C., Weiss, J. N., Chen, P. S., and Lin, S. F. (2009). Intracellular calcium dynamics and acceleration of sinus rhythm by beta-adrenergic stimulation. *Circulation* 119, 788–796.
- Ju, Y. K., and Allen, D. G. (1999). How does beta-adrenergic stimulation increase the heart rate? The role of intracellular Ca^{2+} release in amphibian pacemaker cells. *J. Physiol. (Lond.)* 516(Pt 3), 793–804.
- Ke, Y., Lei, M., Collins, T. P., Rakovic, S., Mattick, P. A., Yamasaki, M., Brodie, M. S., Terrar, D. A., and Solaro, R. J. (2007). Regulation of L-type calcium channel and delayed rectifier potassium channel activity by p21-activated kinase-1 in guinea pig sinoatrial node pacemaker cells. *Circ. Res.* 100, 1317–1327.
- Kharche, S., Yu, J., Lei, M., and Zhang, H. (2011). A mathematical model of action potentials of mouse sinoatrial node cells with molecular bases. *Am. J. Physiol. Heart Circ. Physiol.* 301, H945–H963.
- Kodama, I., Boyett, M. R., Nikmaram, M. R., Yamamoto, M., Honjo, H., and Niwa, R. (1999). Regional differences in effects of E-4031 within the sinoatrial node. *Am. J. Physiol.* 276, H793–H802.
- Kodama, I., Nikmaram, M. R., Boyett, M. R., Suzuki, R., Honjo, H., and Owen, J. M. (1997). Regional differences in the role of the Ca^{2+} and Na^+ currents in pacemaker activity in the sinoatrial node. *Am. J. Physiol.* 272(6 Pt 2), H2793–H2806.
- Kurata, Y., Matsuda, H., Hisatome, I., and Shibamoto, T. (2008). Regional difference in dynamical property of sinoatrial node pacemaking: role of Na^+ channel current. *Biophys. J.* 95, 951–977.
- Lancaster, M. K., Jones, S. A., Harrison, S. M., and Boyett, M. R. (2004). Intracellular Ca^{2+} and pacemaking within the rabbit sinoatrial node: heterogeneity of role and control. *J. Physiol. (Lond.)* 556(Pt 2), 481–494.
- Lei, M., and Brown, H. F. (1996). Two components of the delayed rectifier potassium current, I_{K} , in rabbit sinoatrial node cells. *Exp. Physiol.* 81, 725–741.
- Lei, M., Brown, H. F., and Terrar, D. A. (2000). Modulation of delayed rectifier potassium current, I_{K} , by isoprenaline in rabbit isolated pacemaker cells. *Exp. Physiol.* 85, 27–35.
- Lei, M., Cooper, P. J., Camellitti, P., and Kohl, P. (2002). Role of the 293b-sensitive, slowly activating rectifier potassium current, $\text{i}(\text{K}_{\text{s}})$, in pacemaker activity of rabbit isolated sinoatrial node cells. *Cardiovasc. Res.* 53, 68–79.
- Liao, Z., Lockhead, D., Larson, E. D., and Proenza, C. (2010). Phosphorylation and modulation of hyperpolarization-activated HCN4 channels by protein kinase A in the mouse sinoatrial node. *J. Gen. Physiol.* 136, 247–258.
- Maltsev, V. A., and Lakatta, E. G. (2009). Synergism of coupled subsarcolemmal Ca^{2+} clocks and sarcolemmal voltage clocks confers robust and flexible pacemaker function in a novel pacemaker cell model. *Am. J. Physiol. Heart Circ. Physiol.* 296, H594–H615.
- Nikmaram, M. R., Liu, J., Abdelrahman, M., Dobrzynski, H., Boyett, M. R., and Lei, M. (2008). Characterization of the effects of ryanodine, TTX, E-4031 and 4-AP on the sinoatrial and atrioventricular nodes. *Prog. Biophys. Mol. Biol.* 96, 452–464.
- Noma, A., Kotake, H., and Irisawa, H. (1980). Slow inward current and its role mediating the chronotropic effect of epinephrine in the rabbit sinoatrial node. *Pflugers Arch.* 388, 1–9.
- Ono, K., and Ito, H. (1995). Role of rapidly activating delayed rectifier K^+ current in sinoatrial node pacemaker activity. *Am. J. Physiol.* 269, H453–H462.
- Sato, N., Tanaka, H., Habuchi, Y., and Giles, W. R. (2000). Electrophysiological effects of ibutilide on the delayed rectifier K^+ current in rabbit sinoatrial and atrioventricular node cells. *Eur. J. Pharmacol.* 22, 281–288.
- Shannon, T. R., Wang, F., and Bers, D. M. (2005). Regulation of cardiac sarcoplasmic reticulum Ca release by luminal $[\text{Ca}]$ and altered gating assessed with a mathematical model. *Biophys. J.* 89, 4096–4110.
- Shannon, T. R., Wang, F., Puglisi, J., Weber, C., and Bers, D. M. (2004). A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. *Biophys. J.* 87, 3351–3371.
- Shinagawa, Y. S. H., and Noma, A. (2000). The sustained inward current and inward rectifier K^+ current in pacemaker cells dissociated from rat sinoatrial. *J. Physiol. (Lond.)* 523, 593–605.
- Sosunov, E. A., and Anyukhovsky, E. P. (2012). Differential effects of ivabradine and ryanodine on pacemaker activity in canine sinus node and Purkinje fibers. *J. Cardiovasc. Electrophysiol.* (in press). doi: 10.1111/j.1540-8167.2011.02285.x
- Toyoda, F., Ding, W. G., and Matsumura, H. (2005). Responses of the sustained inward current to autonomic agonists in guinea-pig sinoatrial node pacemaker cells. *Br. J. Pharmacol.* 144, 660–668.
- Vinogradova, T. M., Bogdanov, K. Y., and Lakatta, E. G. (2002). beta-Adrenergic stimulation modulates ryanodine receptor $\text{Ca}(2+)$ release during diastolic depolarization to accelerate pacemaker activity in rabbit sinoatrial nodal cells. *Circ. Res.* 90, 73–79.
- Vinogradova, T. M., Sirenko, S., Lyashkov, A. E., Younes, A., Li, Y., Zhu, W., Yang, D., Ruknudin, A. M., Spurgeon, H., and Lakatta, E. G. (2008). Constitutive phosphodiesterase activity restricts spontaneous beating rate of cardiac pacemaker cells by suppressing local Ca^{2+} releases. *Circ. Res.* 102, 761–769.
- Walsh, K. B., Begenisich, T. B., and Kass, R. S. (1988). Beta-adrenergic modulation in the heart. Independent regulation of K and Ca channels. *Pflugers Arch.* 411, 232–234.
- Wu, Y., Gao, Z., Chen, B., Koval, O. M., Singh, M. V., Guan, X., Hund, T. J., Kutschke, W., Sarma, S., Grumbach, I. M., Wehrens, X. H., Mohler, P. J., Song, L. S., and Anderson, M. E. (2009). Calmodulin kinase II is required for fight or flight sinoatrial node physiology. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5972–5977.
- Yazawa, K., and Kameyama, M. (1990). Mechanism of receptor-mediated modulation of the delayed outward potassium current in guinea-pig ventricular myocytes. *J. Physiol. (Lond.)* 421, 135–150.
- Zaza, A., Robinson, R. B., and DiFrancesco, D. (1996). Basal responses of the L-type Ca^{2+} and hyperpolarization-activated currents to autonomic agonists in the rabbit sinoatrial node. *J. Physiol. (Lond.)* 491(Pt 2), 347–355.
- Zhang, H., Holden, A. V., Kodama, I., Honjo, H., Lei, M., Varghese, T., and Boyett, M. R. (2000). Mathematical models of action potentials in the periphery and center of the rabbit sinoatrial node. *Am. J. Physiol. Heart Circ. Physiol.* 279, H397–H421.

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